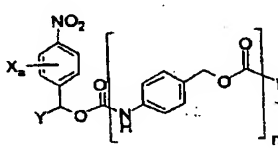
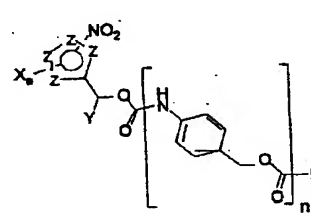


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<p>(54) Title: N-PROTECTED AMINES AND THEIR USE AS PRODRUGS</p> <div style="display: flex; justify-content: space-around; align-items: center;"> <div style="text-align: center;">  <p>(I)</p> </div> <div style="text-align: center;">  <p>(II)</p> </div> </div> <p>(57) Abstract</p> <p>Compounds of formula (I) or (II), wherein X represents H, C₁₋₆ alkyl or C₁₋₆ alkoxy, said alkyl or alkoxy being optionally substituted with one or more groups; a is 0,1,2,3 or 4; Y represents H or C₁₋₆ alkyl; 1, 2 or 3 of the members Z of the 5-membered aromatic ring are independently selected from -O-, -S-, -N= or -NR-, where R is H or C₁₋₆ alkyl optionally substituted with one or more of groups; and E represents a moiety such that EH is an amine; provided that in formula (I) if a = 0 then Y≠H, are provided along with a method of selecting desired protecting groups by measuring the fragmentation rates of compounds of formula (I) or (II) when the nitro group is selected.</p>		

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N-PROTECTED AMINES AND THEIR USE AS PRODRUGS

The present invention relates to methods and compounds for providing amines with N-protecting groups. It further relates to the protected amines themselves and their use as
 5 prodrugs.

The amines are protected as nitroaromatic carbamates (where "aromatic" includes "heteroaromatic"). They include nitro groups which are susceptible to reduction, leading to loss of the protecting group. This is desirably biologically active, the protection. Thus it may be an amine-based or an enediyne. *Evelyna, 13R missing STP*

Thus suitable prodrugs may be useful as prodrugs for directed enzyme prodrug

agents, and/or may be used in ADEPT) or gene-reductase enzymes.

15 BACKGROUND TO THE INVENTION

The use of prodrugs of active compounds in vivo)

is converted into more active compounds, particularly in cancer

therapy. For example a prodrug may be converted into an anti-tumour agent under the influence of an enzyme that is linkable to a monoclonal antibody that will bind to a tumour
 20 associated antigen. The combination of such a prodrug with such an enzyme monoclonal/antibody conjugate represents a very powerful clinical agent. This approach to cancer therapy, often referred to as "antibody directed enzyme/prodrug therapy" (ADEPT), is disclosed in WO88/07378.

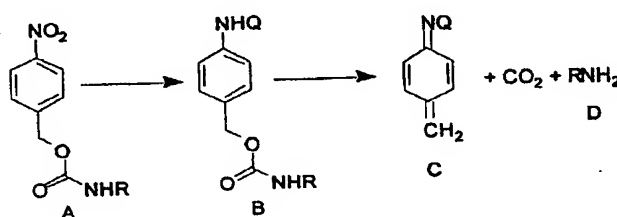
A further therapeutic approach termed "virus-directed enzyme prodrug therapy" (VDEPT) has been proposed as a method for treating tumour cells in patients using prodrugs. Tumour cells are targeted with a viral vector carrying a gene encoding an enzyme capable of activating a prodrug. The gene may be transcriptionally regulated by tissue specific promoter or enhancer sequences. The viral vector enters tumour cells and expresses the enzyme, in order that a prodrug is converted to an active drug within the tumour cells (*Huber et al.*, Proc. Natl. Acad. Sci. USA (1991) 88, 8039). Alternatively, non-viral methods for the delivery of
 30 genes have been used. Such methods include calcium phosphate co-precipitation, microinjection, liposomes, direct DNA uptake, and receptor-mediated DNA transfer. These

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are reviewed in Morgan & French, *Annu. Rev. Biochem.*, 1993, **62**;191. The term "GDEPT" (gene-directed enzyme prodrug therapy) is used to include both viral and non-viral delivery systems.

4-Nitrobenzyl carbamates (A) undergo multi-electron reduction to produce amines.

- 5 The mechanism probably involves the formation of electron-donating 4-hydroxylamine (B; Q=OH) or 4-amine (B; Q=H) species, which then fragment to generate a quinoneimine methide (C) and an amine (D) [P.L. Carl, P.K. Charkravarty, and J.A. Katzenellenbogen, *J. Med. Chem.*, 1981, **24**, 479].

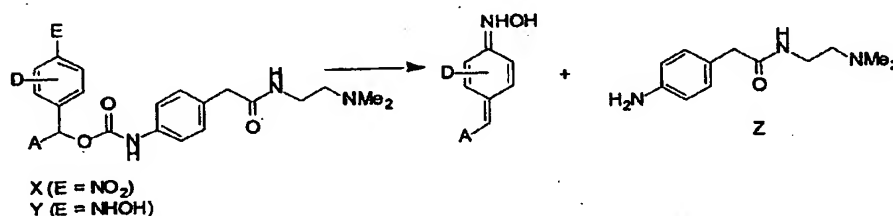


- Despite a low reduction potential (ca. -490 mV) [P. Wardman, *Environ. Health Perspect.*, 1985, **64**, 309] the 4-nitrobenzyl carbamate moiety undergoes facile reduction by the *E. coli* NR enzyme, and has been used as a prodrug "trigger" to deactivate highly cytotoxic amine "effectors" [M.P. Hay and W.A. Denny, *Drugs Future*, 1996, **21**, 917]. The *E. coli* enzyme has been shown to activate 4-nitrobenzyl carbamate derivatives of a limited number of amine-based cytotoxins, including actinomycin D and anthracyclines [A.B. Mauger, P.J. Burke, H.H. Somani, F. Friedlos and R.J. Knox, *J. Med. Chem.*, 1994, **37**, 3452], aniline mustards [A.B. Mauger, P.J. Burke, H.H. Somani, F. Friedlos and R.J. Knox, *J. Med. Chem.*, 1994, **37**, 3452; M. Lee, J.E. Simpson Jr, S. Woo, C. Kaenzig, G.M. Anlezark, E. Eno-Amooquaye, and P.J. Burke, *Bioorg. Med. Chem. Lett.*, 1997, **7**, 1065] and enediynes [M.P. Hay, W.R. Wilson, and W.A. Denny, *Bioorg. Med. Chem. Lett.*, 1995, **5**, 2829]. All of these studies have used the otherwise unsubstituted 4-nitrobenzyl carbamate moiety.

- To be fully effective, such prodrugs must be activated efficiently by the enzyme, and the resulting reduced species must fragment rapidly to release the cytotoxic amine effector. Kinetic structure-activity relationships (SAR) have been extensively studied for the one-electron reduction of nitrobenzyl halides [D.L. Kirkpatrick, K.E. Johnson, and A.C. Sartorelli, *J. Med. Chem.*, 1986, **29**, 2048] and quaternary salts [M. Tercel, W.R. Wilson,

R.F. Anderson, and W.A. Denny, *J. Med. Chem.*, 1996, **39**, 1084 and refs therein], but not for 4-nitrobenzyl carbamates. We have found that suitable substituents on the 4-nitrobenzyl ring and/or alpha-carbon result in more rapid fragmentation of the 4-hydroxylamine intermediates, and can also serve as sites for attaching solubilising functionalities.

- 5 For a series of substituted 4-nitrobenzyl carbamate model compounds (X), fragmentation rates of the corresponding 4-hydroxylamines (Y) to release amines (Z) correlated with electron-donating properties (σ_p) of the substituent, as shown in Table 1. The maximum half-lives ($Mt_{1/2}$) of the hydroxylamine derivatives were measured by HPLC, following 4-fold stoichiometry radiolytic reduction of the corresponding substituted 4-
- 10 nitrobenzyl carbamates. Assuming first order conditions, the half-life ($t_{1/2}$) of species R is calculated from the equation $\ln([R]_0/[R]_t) = t(\ln 2/t_{1/2})$. The ratio $[R]_0/[R]_t$ was taken as the fraction of nitrobenzyl carbamate which had not released the amine (Z) after 4-fold reduction. This method yields a maximum value for the half-life of fragmentation.



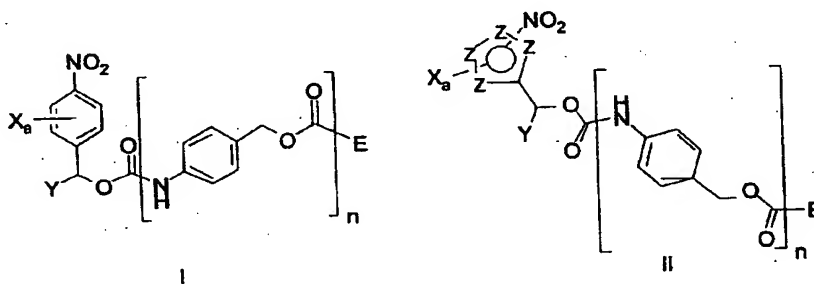
- Table 1 Half-lives for fragmentation ($Mt_{1/2}$) and percent of amine released (t_0) for substituted 4-hydroxylaminobenzyl carbamates (derived from the corresponding 4-
- 15 nitrobenzyl carbamates by radiolytic reduction).

D	A	σ_p	Mt _{1/2} (min)	t ₀ (%)
2-NO ₂	H	0.78	88	18
3-NO ₂	H	0.71	65	22
3-CO ₂ Me	H	0.37	20	44
3-OMe	H	0.12	17	37
H	H	0.0	16	40
2-OMe	H	-0.27	12	48
2-NHMe	H	-0.84	7	65
H	Me	0.0	9.5	-

Table 1 shows that the unsubstituted hydroxylaminobenzyl carbamate normally used as a trigger has a half-life of 16 minutes. This is relatively long and, under biological conditions, may result in substantial loss of material by side reactions not involving (activating) amine release. The half-life can be lowered significantly by the use of electron-donating substituents, and/or by the use of α -substituents (A).

DISCLOSURE OF THE INVENTION

In a first aspect, the invention provides a method of providing an amine with a protecting group comprising (i) providing a plurality of different compounds selected from compounds of formulae (I) and (II)



wherein:

X represents H, C₁₋₆ alkyl or C₁₋₆ alkoxy, said alkyl or alkoxy being optionally substituted with one or more of the following groups: hydroxy (OH), ether (OR_x), amino (NH₂), mono-substituted amino (NR_xH), di-substituted amino (NR_x¹R_x²), cyclic C₁₋₅ alkylamino, imidazolyl, C₁₋₆ alkylpiperazinyl, morpholino, thiol (SH), thioether (SR_x), tetrazole, carboxy (COOH), carboxylate (COOR_x), sulphony (S(=O)₂OH), sulphonate

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- (S(=O)₂OR_x), sulphonyl (S(=O)₂R_x), sulphixy (S(=O)OH), sulphinate (S(=O)OR_x), sulphinyl (S(=O)R_x), phosphonoxy (OP(=O)(OH)₂) and phosphate (OP(=O)(OR_x)₂), where R_x, R_x¹ and R_x² are selected from a C₁₋₆ alkyl group, a C₃₋₂₀ heterocyclyl group or a C₅₋₂₀ aryl group, preferably a C₁₋₆ alkyl group; a is 0, 1, 2, 3 or 4; Y represents H or C₁₋₆ alkyl; 1, 2 or 3 of the members Z of the 5-membered aromatic ring are independently selected from -O-, -S-, -N= or -NR-, (where R is H or C₁₋₆ alkyl optionally substituted with one or more of the following groups: hydroxy (OH), ether (OR_R), amino (NH₂), mono-substituted amino (NR_RH), di-substituted amino (NR_R¹R_R²), C₁₋₅ cyclic amino, imidazolyl, alkylpiperazinyl, morpholino, thiol (SH), thioether (SR_R), tetrazole, carboxy (COOH), carboxylate (COOR_R), sulphonyl (S(=O)₂OH), sulphonate (S(=O)₂OR_R), sulphonyl (S(=O)₂R_R), sulphixy (S(=O)OH), sulphinate (S(=O)OR_R), sulphinyl (S(=O)R_R), phosphonoxy (OP(=O)(OH)₂) and phosphate (OP(=O)(OR_R)₂), where R_R, R_R¹ and R_R² are selected from a C₁₋₆ alkyl group, a C₃₋₂₀ heterocyclyl group or a C₅₋₂₀ aryl group, preferably a C₁₋₆ alkyl group), the other ring atoms being C; n is 0 or 1; and E represents a moiety such that EH is an amine;
- 15 (ii) measuring the rates of fragmentation of the compounds to release EH when the nitro group is reduced and selecting a compound having a desired rate of decomposition; and
- (iii) providing the amine to be protected with a protecting group corresponding to that in the selected compound.

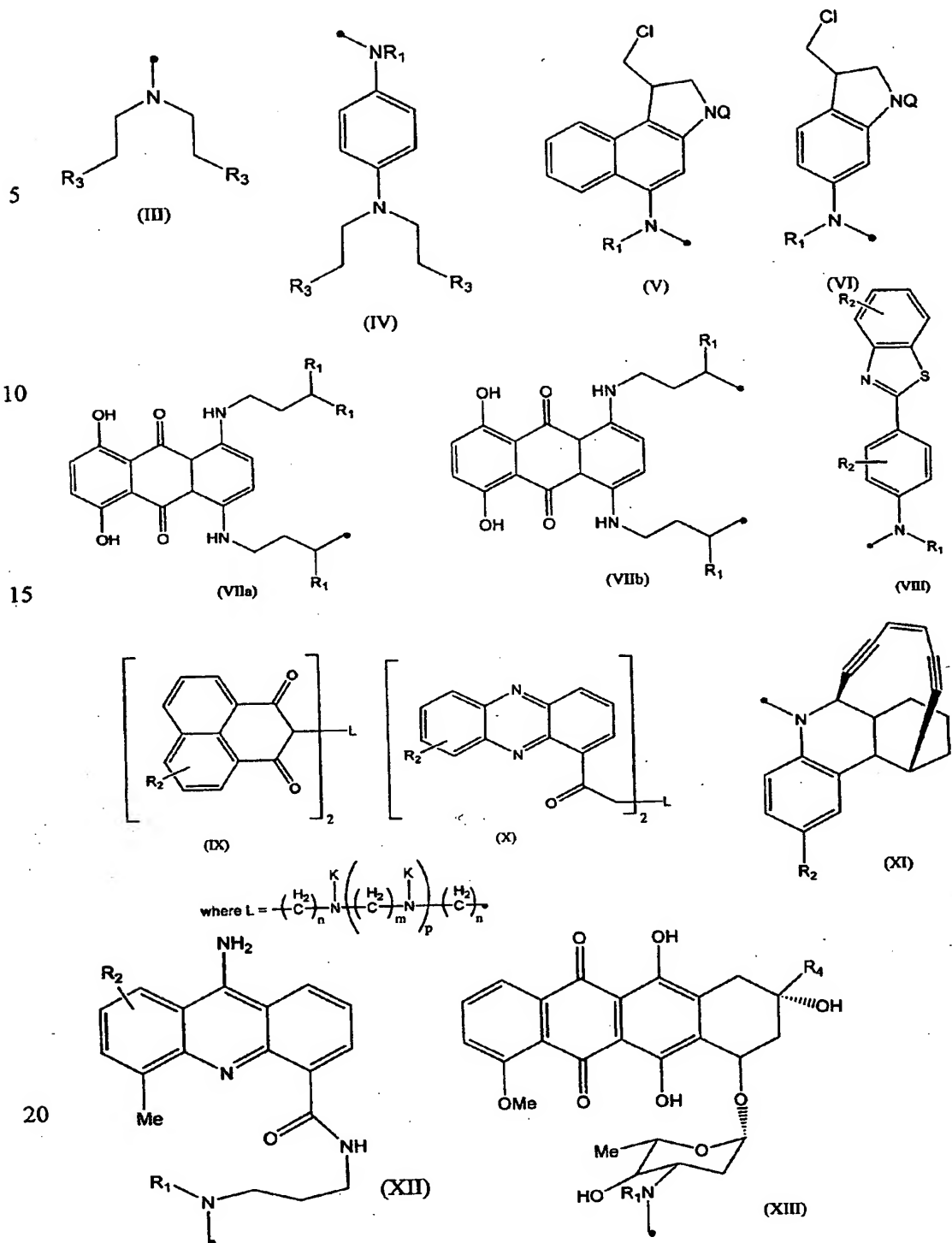
- In this aspect, the step of selecting the compound is preferably carried out in order to
- 20 provide a protecting group with a faster rate of fragmentation than unsubstituted 4-nitrobenzyl carbamate. However selecting a compound bearing a protecting group with a slower rate of fragmentation than 4-nitrobenzyl carbamate may be preferred. This particularly applies in situations in which it is desired to provide a prodrug which can diffuse away from the site of actuation by the appropriate enzyme, and thus kill tumour cells further away from the site of
- 25 actuation (the "bystander" effect).

In a second aspect, the present invention relates to a compound represented by the general formula (I) or (II) as shown above, wherein X, Y, Z, E, a and n are as defined above; provided that in formula (I) if a = 0 then Y ≠ H.

EH is preferably a cytotoxic amine. E may be selected from formulae (III-XIII).

- 30 In (III-XIII), R₁ represents H or C₁₋₆ alkyl, being optionally substituted with one or more of the following groups: one or more of the following groups: hydroxy (OH), ether (OR_E), amino (NH₂), mono-substituted amino (NR_EH), di-substituted amino (NR_E¹R_E²), cyclic

- C_{1-5} alkylamino, imidazolyl, C_{1-6} alkylpiperazinyl, morpholino, thiol (SH), thioether (SR_E), tetrazole, carboxy (COOH), carboxylate ($COOR_E$), sulphony ($S(=O)_2OH$), sulphonate ($S(=O)_2OR_E$), sulphonyl ($S(=O)_2R_E$), sulphoxy ($S(=O)OH$), sulphinate ($S(=O)OR_E$), sulphinyl ($S(=O)R_E$), phosphonooxy ($OP(=O)(OH)_2$) and phosphate ($OP(=O)(OR_E)_2$), where R_E , R_E^1 and R_E^2 are selected from a C_{1-6} alkyl group, a C_{3-20} heterocyclyl group or a C_{5-20} aryl group, more preferably from a C_{1-6} alkyl group; R_2 represents H, C_{1-6} alkyl, C_{1-6} alkoxy, OH, halogen, NO_2 , NH_2 , $NHMe$, NMe_2 , SO_2Me , CF_3 , CN, $CONH_2$ or $CONHMe$; each R_3 is independently selected from Cl, Br, I and OMS; and R_4 is selected from $-C(=O)Me$ and $-C(=O)CH_2OH$; Q represents substituted indole, substituted benzofuran or substituted cinnamoyl; in (IX) and (X), each n is independently from 2-4, and each m is independently from 2-4, and p = 0 or 1.



Compounds of formula V are described in EP 0 938 474, which is incorporated herein by reference. Compounds of formula VI are described in EP 0 850 220, which is incorporated herein by reference.

A compound of formula (I) or (II) may be basic or acidic and may thus form pharmaceutically acceptable salts with both organic and inorganic acids and bases. These are included within the scope of the second aspect.

In a first type of preferred embodiment, X represents C_{1-6} alkyl or C_{1-6} alkoxy, said alkyl or alkoxy being optionally substituted with one or more of the following groups: hydroxy, ether (OR_x), amino, alkylamino (NR_xH), dialkylamino ($NR_x^1R_x^2$), cyclic C_{1-5} alkylamino, imidazolyl, C_{1-6} alkylpiperazinyl, morpholino, thiol, alkylthioether (SR_x), tetrazole and $-CO_2X'$ where X' is selected from the possibilities listed for X and R_x , R_x^1 and R_x^2 are selected from C_{1-6} alkyl; a is 0, 1, 2, 3 or 4; Y represents H or lower alkyl; 1, 2 or 3 of the members Z of the 5-membered aromatic ring are independently selected from -O-, -S-, -N= or -NR-, (where R is H or lower alkyl optionally substituted with one or more of the following groups: hydroxy, ether (OR_R), amino, alkylamino (NR_RH), dialkylamino ($NR_R^1R_R^2$), cyclic C_{1-5} alkylamino, imidazolyl, C_{1-6} alkylpiperazinyl, morpholino, thiol, alkylthioether (SR_R), tetrazole and $-CO_2R'$ where R' is selected from the possibilities listed for R and R_R , R_R^1 and R_R^2 are selected from C_{1-6} alkyl); and E represents R_1 , represents H or C_{1-6} alkyl, being optionally substituted with one or more of the following groups hydroxy, ether (OR_E), amino, alkylamino (NR_EH), dialkylamino ($NR_E^1R_E^2$), cyclic C_{1-5} alkylamino, imidazolyl, C_{1-6} alkylpiperazinyl, morpholino, thiol, alkylthioether (SR_E), tetrazole and $-CO_2X'$ where X' is selected from the possibilities listed for X and R_E , R_E^1 and R_E^2 are selected from C_{1-6} alkyl.

In a second type of preferred embodiment, the compound is of formula (I) where a is

1. It is further preferred in this embodiment that X represents optionally substituted C_{1-6} alkoxy and more preferably in the 2 position. The alkoxy group is preferably selected from methyl, ethyl and n-propyl, and the substituents from hydroxy, methoxy, phosphonoxy, NMe_2 , $Nmorph$, OCO_2-tBu , and OCO_2H . In more preferred embodiments the ethyl or n-propyl group is singly substituted, most preferably with hydroxy, whereas the Me group is unsubstituted.

In this type of embodiment Y is preferably selected from H or Me. E is preferably selected from III - XIII, most preferably V or XIII. If E is selected from XIII, n is preferably

1.

In a third preferred type of embodiment, the compound is of formula (II) with one Z

being -N= and another Z being -NR-, R being preferably Me or Et, the other members of the heterocyclic ring being C. If R is Et, it is preferably substituted with hydroxy. A is preferably 0. More preferably the -N= and -NR- are not adjacent in the heterocyclic ring. The most preferred arrangement is 2 (-NMe-) and 5 (-N=), with the NO₂ at the 3 position. In this type of embodiment, Y is preferably selected from H or Me. E is preferably selected from V or XIII. If E is selected from XIII, then n is preferably 1.

In a fourth preferred type of embodiment, the compound is of formula (II), a is 0, and Z is either O or S. It is further preferred that the O or S is in the 2 position in the ring, and the NO₂ is attached to the 3 position. In this type of embodiment, Y is preferably selected from H or Me.

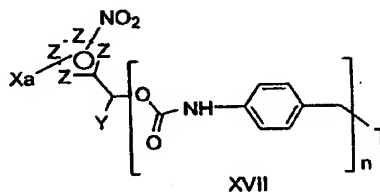
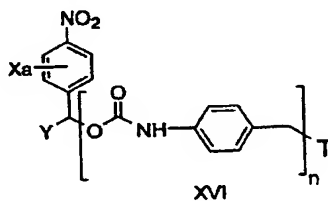
In a fifth preferred embodiment, the compound is of formula (II), and Z is NR, preferably NMe. There may be one further substituent (a=1), or there may be no further substituents on the ring (a=0). The further substituent is preferably CO₂Et. In this type of embodiment, Y is preferably selected from H or Me.

In a third aspect the invention provides a compound according to the second aspect for pharmaceutical use.

In a fourth aspect the invention provides the use of a compound according to the second aspect for the manufacture of a composition for use in the treatment of a hyper-proliferative disease, particularly a neoplastic disease. The composition may also include activating means for simultaneous or separate administration, the activating means typically comprising an enzyme or means for providing an enzyme, for performing ADEPT or VDEPT therapy. The activating means typically leads to liberation of the amine EH.

In a fifth aspect the invention provides a compound of the formula XVI or XVII where X, n, Z and Y are as defined for the second aspect and T is OH or an activated alcohol functionality (such as -O.CO.L where L is a leaving group such as Cl) suitable for reaction with an amine EH to produce a compound according to the second aspect.

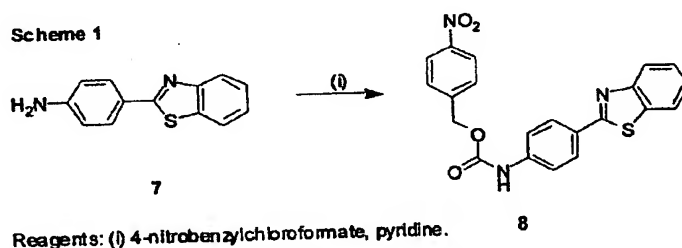
In a sixth aspect the invention provides the use of a compound of formula (XVI) or



(XVII) in protecting an amine. This may include activation of an alcohol (XVI or XVII where T is OH) with a reagent such as phosgene, diphosgene or triphosgene or a chloroformate, e.g. 4-nitrobenzylchloroformate or pentafluorophenylchloroformate, optionally in conjunction with HOBT(1-hydroxybenzotriazole).

In a further aspect, the present invention relates to a method of preparing compounds of the general formula (I); examples of the methods are outlined in Schemes 1-24.

Thus (Scheme 1), reaction of the amine 7 [D-F. Shi, T. D. Bradshaw, S. Wrigley, C. J. McCall, P. Lelieveld, I. Fitchner, M. F. G. Stevens. *J. Med. Chem.*, 1996, 39, 3375] with 4-nitrobenzylchloroformate gave carbamate 8.



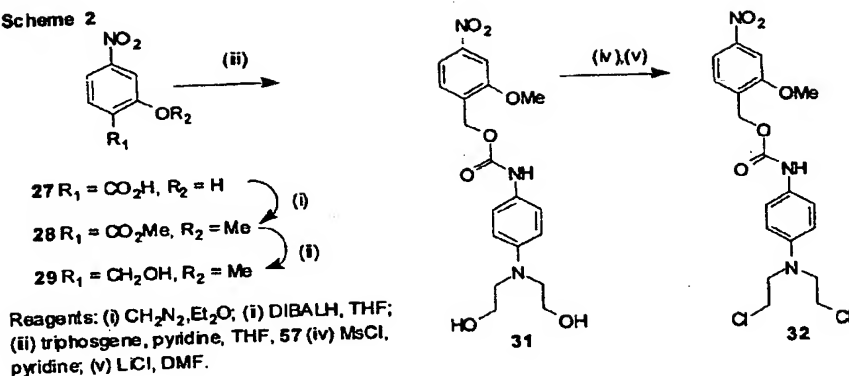
Reaction of the 1,4-difluoro-5,8-dihydroxyanthracene-9,10-dione 22 with amine 15 in pyridine gave the monocarbamate 23 and biscarbamate 24 (Scheme 7). Further reaction of 23 with 2-(2-aminoethylamino)ethanol gave carbamate 26.

15 In another example (Scheme 2), 4-nitrosalicylic acid (27) was methylated using a solution of diazomethane in ether and the methyl ester 28 reduced with DIBALH in THF to give the nitrobenzyl alcohol 29. Activation of the alcohol 29 with triphosgene (or alternatively phosgene or diphosgene) in the presence of pyridine, and reaction with *N,N'*-bis(2-hydroxyethyl)-1,4-benzenediamine (57) gives the carbamate 31, which was elaborated to the

20 mustard 32 using standard methods.

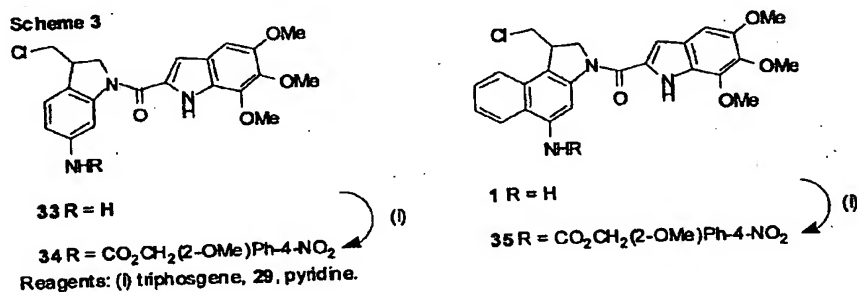
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Scheme 2



Similarly (Scheme 3), activation of alcohol 29 with triphosgene in the presence of pyridine and reaction with amine 33 [M. Tercel and W. A. Denny, *J. Chem. Soc. Perkin Trans. I*, 1998, 509] or amine 1 [G. J. Atwell, W. R. Wilson, W. A. Denny, *Bioorg. Med. Chem. Lett.*, 1997, 7, 1483] gave carbamates 34 and 35, respectively.

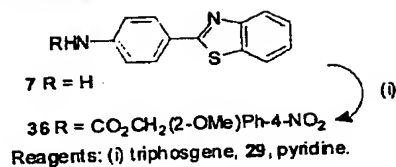
Scheme 3



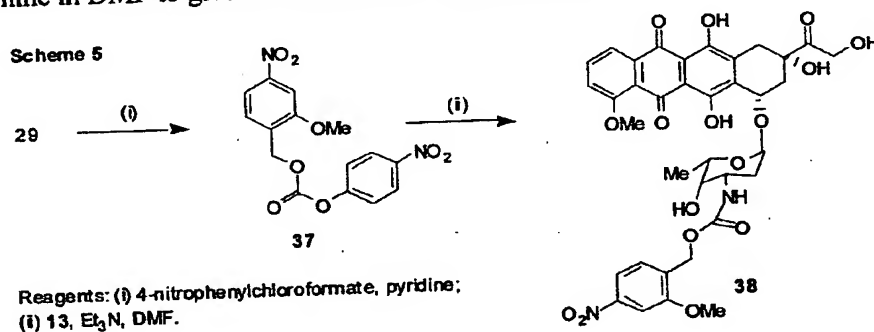
Similarly (Scheme 4), activation of alcohol 29 with triphosgene in the presence of pyridine and reaction with amine 7 [D-F. Shi, T. D. Bradshaw, S. Wrigley, C. J. McCall, P. Lelieveld, I. Fitchner, M. F. G. Stevens, *J. Med. Chem.*, 1996, 39, 3375] gave carbamate 36.

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Scheme 4



In another example (Scheme 5), activation of alcohol 29 with 4-nitrophenylchloroformate gave the carbonate 37 which was reacted with doxorubicin (13) and triethylamine in DMF to give the doxorubicin carbamate 38.

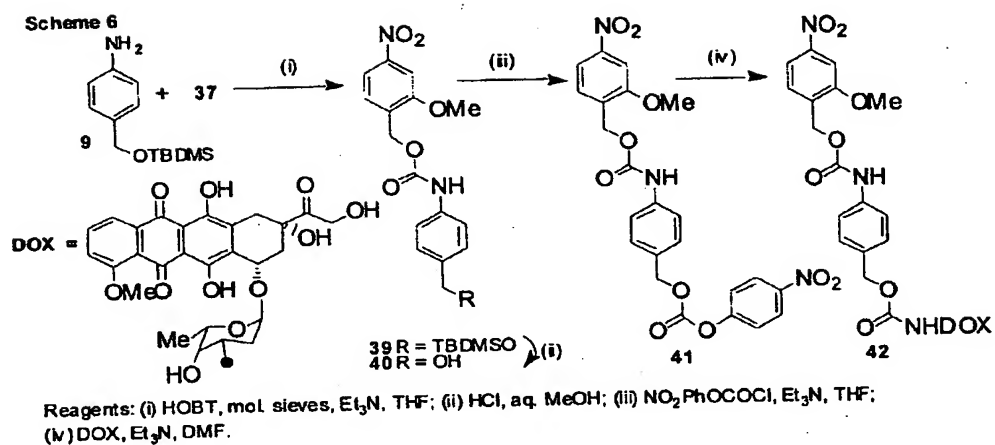


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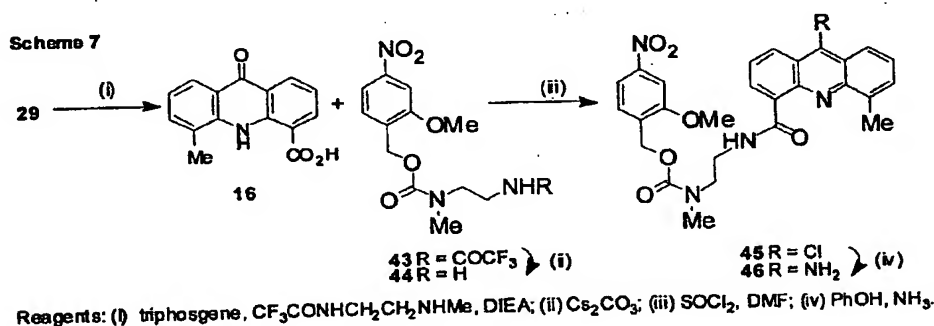
In another example (Scheme 6), carbonate 37 was coupled to amine 9 using 1-hydroxybenzotriazole (HOBt), 4Å molecular sieves and triethylamine to give protected carbamate 39. Removal of the TBDMS protecting group with aqueous acid gave the alcohol 40 which was activated with 4-nitrophenylchloroformate to give the carbonate 41. Reaction of

10 41 with doxorubicin (13) and triethylamine in DMF gave the carbamate 42.

- 13 -

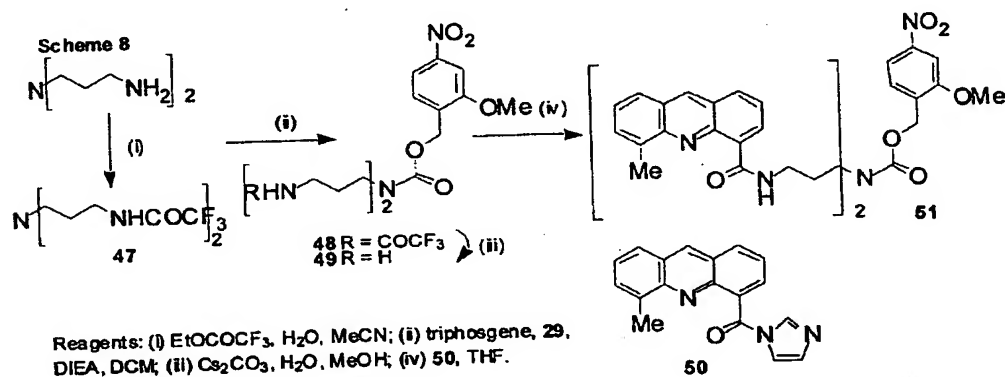


In another example (Scheme 7), reaction of alcohol **29** with triphosgene and triethylamine, and coupling to 2,2,2-trifluoro-*N*-[2-(methylamino)ethyl]acetamide trifluoroacetate gave the trifluoroacetamide **43** which was deprotected under basic conditions to give amine **44**. Activation of the 5-methyl-9-oxo-9,10-dihydro-4-acridinecarboxylic acid (**16**) with thionyl chloride and coupling of the intermediate 9-chloroacridinyl acid chloride with amine **44** gave amide **45** which was converted to carbamate **46** using ammonia in phenol.



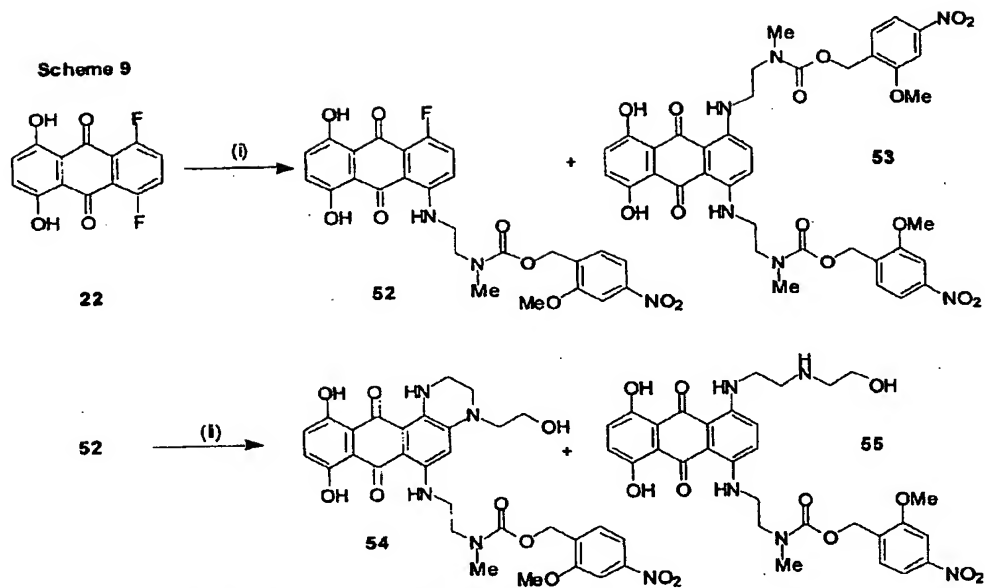
- 14 -

In another example (Scheme 8), the bistrifluoroacetamide **47** was coupled to alcohol **29** to give bisamide **48** which was deprotected under basic conditions to give the amine **49**. The amine **49** was coupled to 4-(1*H*-imidazol-1-ylcarbonyl)-5-methylacridine (**50**) [S. A. Gamage, J. A. Spicer, G. J. Atwell, G. J. Finlay, B. C. Baguley, W. A. Denny, *J. Med. Chem.*, 1999, 42, 2383-2393] to give the carbamate **51**.



Reaction of the 1,4-difluoro-5,8-dihydroxyanthracene-9,10-dione **22** with amine **44** gave the monocarbamate **52** and biscarbamate **53** (Scheme 9). Further reaction of **52** with 2-(2-aminoethylamino)ethanol gave carbamate **55**.

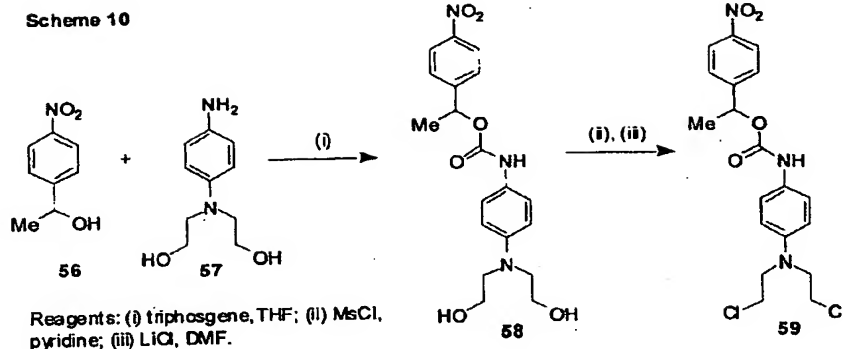
- 15 -



Reagents: (i) 44, pyridine; (ii) $\text{NH}_2(\text{CH}_2)_2\text{NH}(\text{CH}_2)_2\text{OH}$, pyridine.

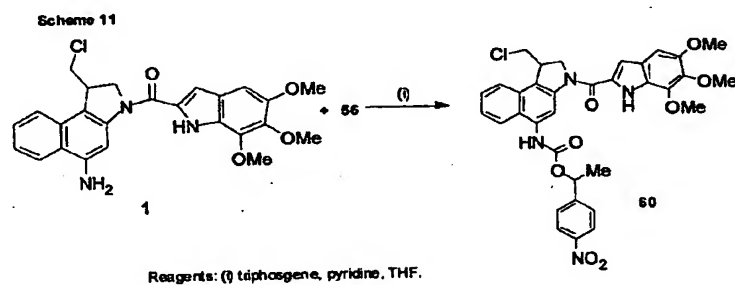
In another example (Scheme 10), reaction of 4-nitrophenylethan-1-ol (56) with triphosgene and pyridine, with the subsequent addition of *N*¹,*N*¹-bis(2-hydroxyethyl)-1,4-benzenediamine (57) gave the carbamate 58 which was elaborated to the dichloride 59.

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In another example (Scheme 11), coupling of the alcohol 56 with amine 1 [G. J. Atwell, W. R. Wilson, W. A. Denny, *Bioorg. Med. Chem. Lett.*, 1997, 7, 1483] using triphosgene and pyridine in THF gave the carbamate 60.

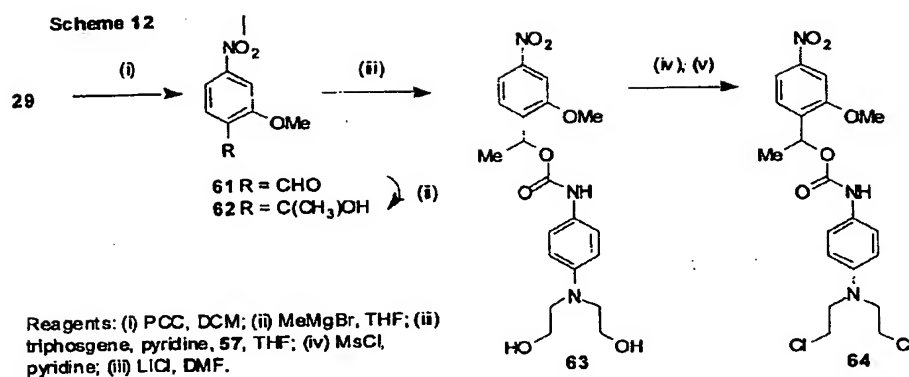
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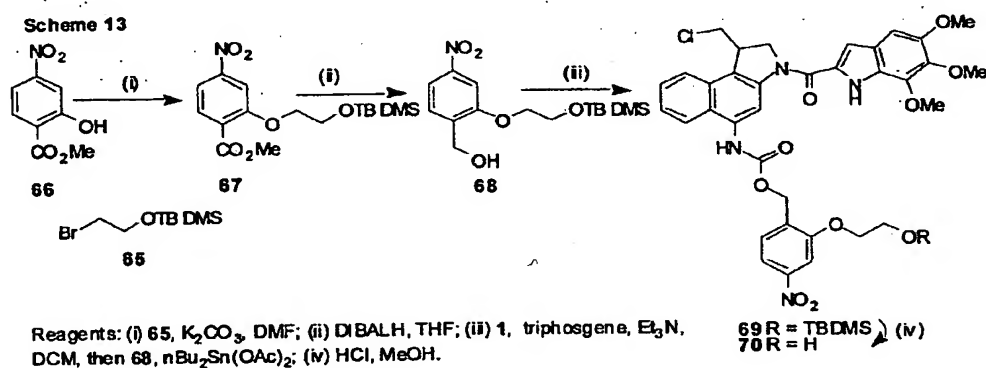
Oxidation of alcohol 29 with pyridinium chlorochromate (PCC) in DCM gave the aldehyde 61 (Scheme 12). Reaction of aldehyde 61 with methyl magnesium bromide in THF gave the alcohol 62 which was coupled to amine 57 to give carbamate diol 63. The diol 63 was

10 elaborated to the dichloride 64 using standard methods.

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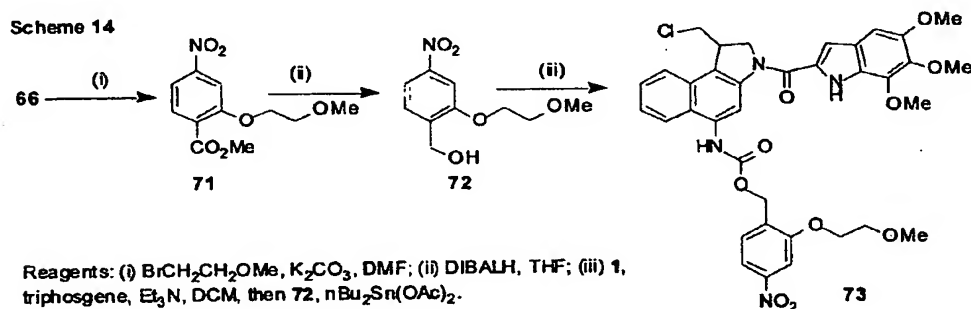


- In another example (Scheme 13), alkylation of methyl 2-hydroxy-4-nitrobenzoate **66** with bromide **65** under basic conditions gave the ester **67** which was reduced to alcohol **68** using DIBALH in THF. Activation of amine **1** [G. J. Atwell, W. R. Wilson, W. A. Denny, *Bioorg. Med. Chem. Lett.*, 1997, 7, 1483] with triphosgene and triethylamine gave an intermediate isocyanate which was coupled with alcohol **68** using catalytic dibutyltin diacetate to give carbamate **69**. Deprotection of **69** under acidic conditions gave **70**.

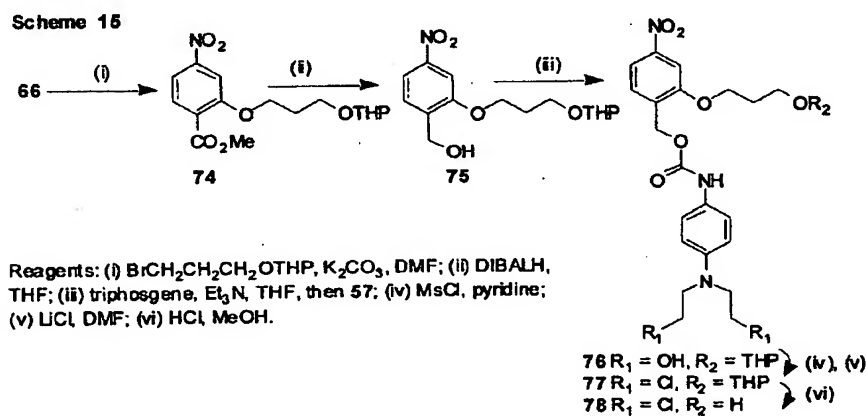


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Similarly (Scheme 14), reaction of phenol 66 with 2-bromoethyl methyl ether gave ester 71 which was reduced to alcohol 72. Activation of amine 1 [G. J. Atwell, W. R. Wilson, W. A. Denny, *Bioorg. Med. Chem. Lett.*, 1997, 7, 1483] with triphosgene and triethylamine gave an intermediate isocyanate which was coupled with alcohol 72 using catalytic dibutyltin diacetate to give carbamate 73.



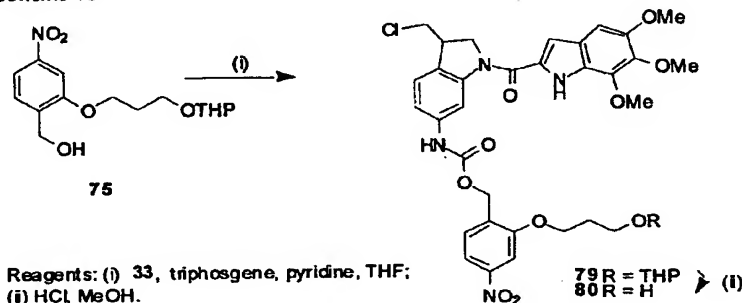
In another example (Scheme 15), phenol 66 was alkylated with 3-iodopropyl tetrahydropyranyl ether under basic conditions to give ester 74 which was reduced to alcohol 75 using DIBALH in THF. Activation of the alcohol 75 with triphosgene and triethylamine (or pyridine, or another organic base) in THF and subsequent reaction with amine 57 gave the carbamate diol 76. The diol 76 was converted to the dichloride 77 using standard methods and the tetrahydropyranyl ether deprotected under acidic conditions to give carbamate 78.



In another example (Scheme 16), alcohol 75 was activated using triphosgene and triethylamine (or pyridine, or another organic base) and coupled to amine 33 [M. Tercel and W. A. Denny, *J. Chem. Soc. Perkin Trans. 1*, 1998, 509] to give carbamate 79 which was deprotected under acidic conditions to give 80.

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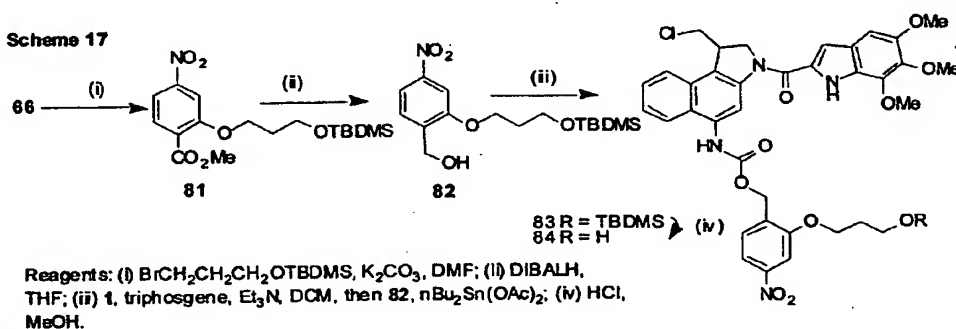
Scheme 16



In another example (Scheme 17), alkylation of phenol 66 with 3-bromopropyl *tert*-butyl(dimethyl)silyl ether under basic conditions gave the ester 81 which was reduced to alcohol 82 using DIBALH in THF. Activation of amine 1 [G. J. Atwell, W. R. Wilson, W. A.

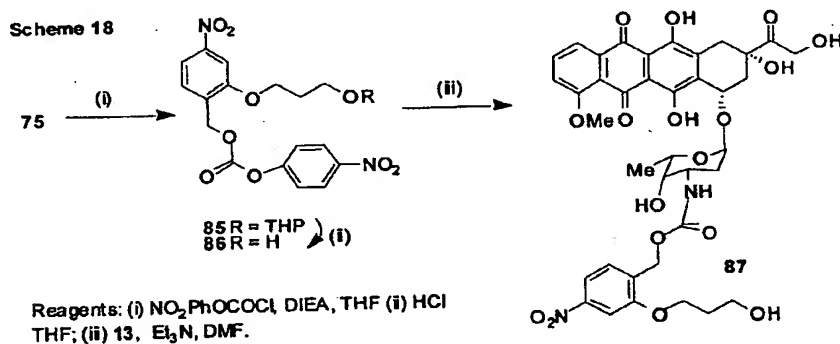
10 Denny, *Bioorg. Med. Chem. Lett.*, 1997, 7, 1483] with triphosgene and triethylamine gave an intermediate isocyanate which was coupled with alcohol 82 using catalytic dibutyltin diacetate to give carbamate 83. Deprotection of 83 under acidic conditions gave carbamate 84.

Scheme 17

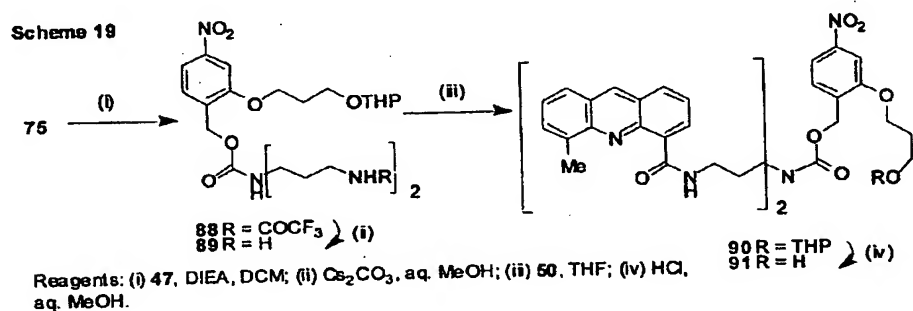


- 20 -

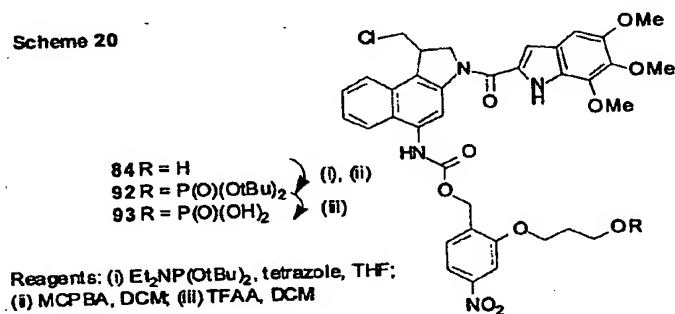
In another example (Scheme 18), activation of alcohol 75 with 4-nitrophenylchloroformate gave the carbonate 37 which was deprotected under acidic conditions to give carbonate 86. Reaction of 86 with doxorubicin (13) and triethylamine in DMF gave the doxorubicin carbamate 87.



In another example (Scheme 19) alcohol 75 was activated with triphosgene and coupled to amine 47 to give trifluoroacetamide 88 which was deprotected under basic conditions to give bis-amine 89. Coupling of the bis-amine 89 with the imidazolidine 50 [S. A. Gamage, J. A. Spicer, G. J. Atwell, G. J. Finlay, B. C. Baguley, W. A. Denny, *J. Med. Chem.*, 1999, 42, 2383-2393] gave the carbamate 90 which was deprotected under acidic conditions to give carbamate 91.

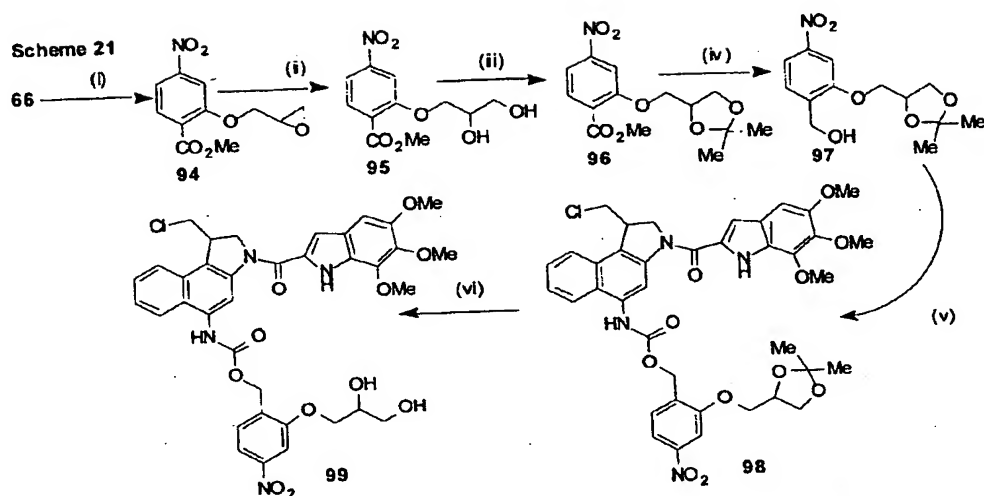


In another example (Scheme 20), the alcohol 84 was reacted with di-*tert*-butyl diethylphosphoramidite and tetrazole in THF and the intermediate oxidised with MCPBA to give ester 92. Deprotection of 92 with trifluoroacetic acid (TFAA) gave the phosphate 93.



In another example (Scheme 21), phenol 66 was alkylated with epichlorohydrin under basic conditions to give epoxide 94. Hydrolysis of 94 with perchloric acid gave diol 95 which was protected as the acetonide 96. Reduction of 96 with DIBALH in THF gave the alcohol 97. Activation of amine 1 [G. J. Atwell, W. R. Wilson, W. A. Denny, *Bioorg. Med. Chem. Lett.*, 1997, 7, 1483] with triphosgene and triethylamine gave an intermediate isocyanate which was coupled with alcohol 97 using catalytic dibutyltin diacetate to give carbamate 98. Deprotection of 98 under acidic conditions gave carbamate 99.

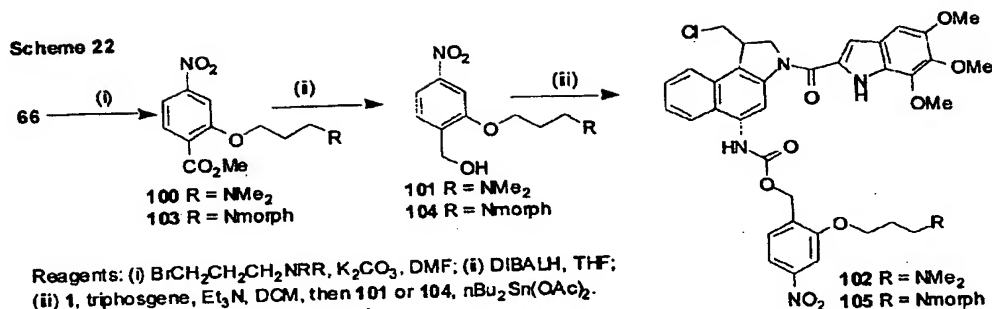
- 22 -



In another example (Scheme 22), phenol 66 was alkylated with *N*-(3-chloropropyl)-*N,N*-dimethylamine under basic conditions to give amine 100. Reduction of 100 with DIBALH in THF gave the alcohol 101. Activation of amine 1 [G. J. Atwell, W. R. Wilson, W. A. Denny, *Bioorg. Med. Chem. Lett.*, 1997, 7, 1483] with triphosgene and triethylamine gave an intermediate isocyanate which was coupled with alcohol 101 using catalytic dibutyltin diacetate to give carbamate 102.

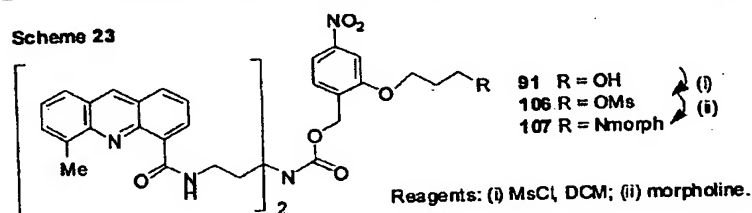
Similarly (Scheme 22), phenol 66 was alkylated with 4-(3-chloropropyl)morpholine under basic conditions to give amine 103. Reduction of 103 with DIBALH in THF gave the alcohol 104. Activation of amine 1 [G. J. Atwell, W. R. Wilson, W. A. Denny, *Bioorg. Med. Chem. Lett.*, 1997, 7, 1483] with triphosgene and triethylamine gave an intermediate isocyanate which was coupled with alcohol 104 using catalytic dibutyltin diacetate to give carbamate 105.

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5

In another example (Scheme 23), reaction of alcohol **91** with methanesulphonyl chloride gave the mesylate **106** which was reacted with morpholine to give carbamate **107**.

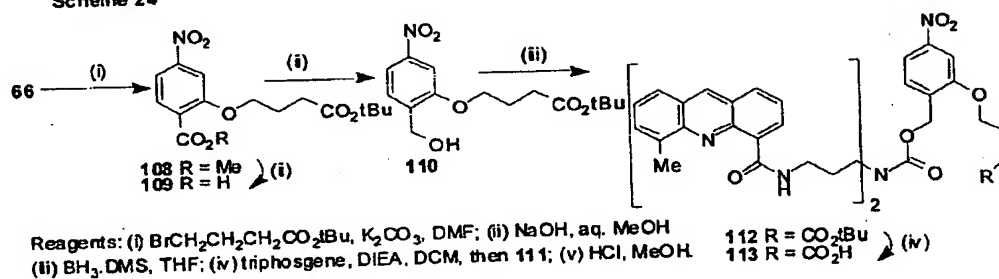


10

In another example (Scheme 24), phenol **66** was alkylated with *tert*-butyl 4-bromobutanoate under basic conditions to give ester **108**. Hydrolysis of **108** under basic conditions gave acid **109** which was reduced with borane.dimethylsulfide in THF to give alcohol **110**. Activation of alcohol **110** with triphosgene and diisopropylethylamine and subsequent coupling with *N,N*-bis[3-(5-methylacridine-4-carboxamido)propyl]amine (**111**) [S. A. Gamage, J. A. Spicer, G. J. Atwell, G. J. Finlay, B. C. Baguley, W. A. Denny, *J. Med.*

Chem., 1999, 42, 2383-2393] gave carbamate 112. Carbamate 112 was deprotected under acidic conditions to give acid 113.

Scheme 24

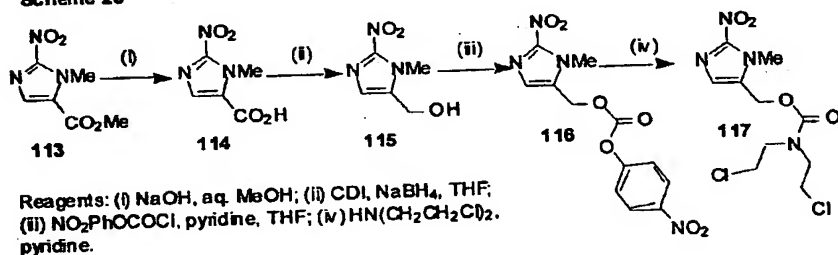


5 In a further aspect, the present invention relates to a method of preparing compounds of the general formula (II); examples of the methods are outlined in Schemes 25-.

Thus (Scheme 25), (2-nitro-1*H*-imidazol-5-yl)methanol (115) is obtained from the known ethyl 2-nitro-1*H*-imidazol-5-ylcarboxylate (113) [B. Cavalleri, R. Ballotta, G.C. Lancini. *J. Heterocyclic Chem.* 1972, 9, 979.] by basic hydrolysis to the acid 114 and

10 reduction of an intermediate imidazolidine with sodium borohydride. This procedure is a major improvement upon the above published methods. Reaction of 115 with 4-nitrophenyl chloroformate gives the activated carbonate 116 which reacts with *N,N*-bis-(2-chloroethyl)amine to give carbamate 117.

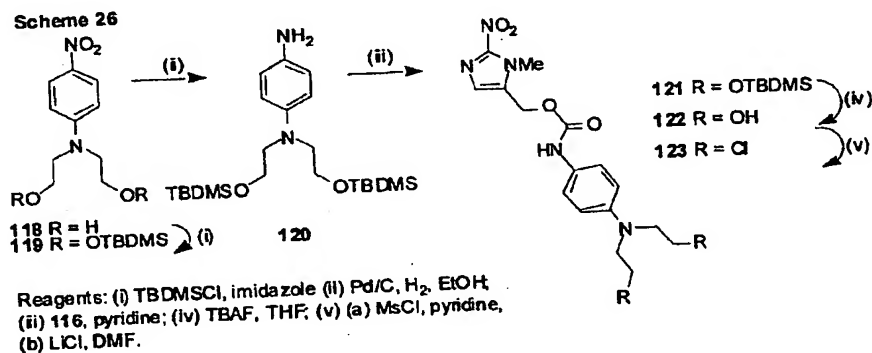
Scheme 25



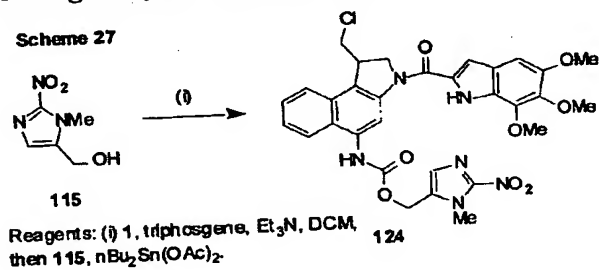
- 25 -

Similarly (Scheme 26), reaction of **116** with the protected phenyldiamine diol **120**, derived from the nitrophenylamino diol **118**, gives carbamate **121**. Deprotection of the bis-silyl alcohol **121** with TBAF gives the diol **122** which can be converted to the dichloride **123** under standard conditions.

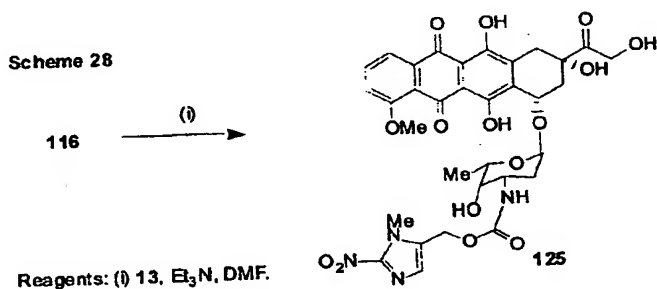
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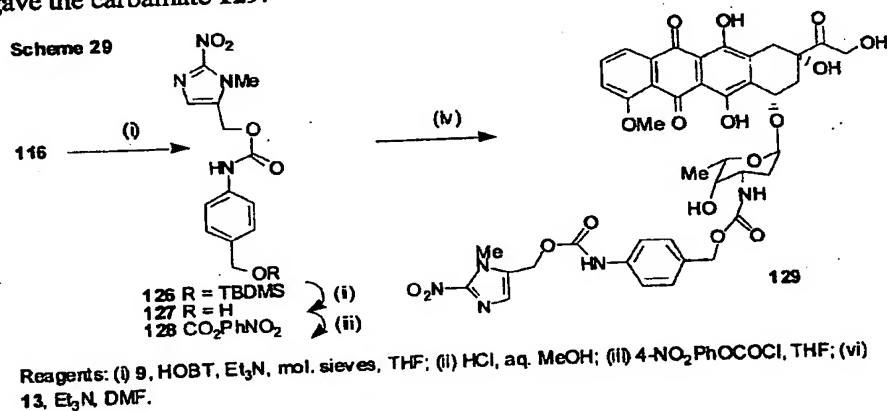
- In another example (Scheme 27), activation of amine **1** [G. J. Atwell, W. R. Wilson, W. A. Denny, *Bioorg. Med. Chem. Lett.*, 1997, 7, 1483] with triphosgene and triethylamine (or pyridine, or another organic base) gave an intermediate isocyanate which was coupled with alcohol **115** using catalytic dibutyltin diacetate to give carbamate **124**.



In another example (Scheme 28), reaction of the carbonate 116 with doxorubicin (13) and triethylamine in DMF gave the carbamate 125.

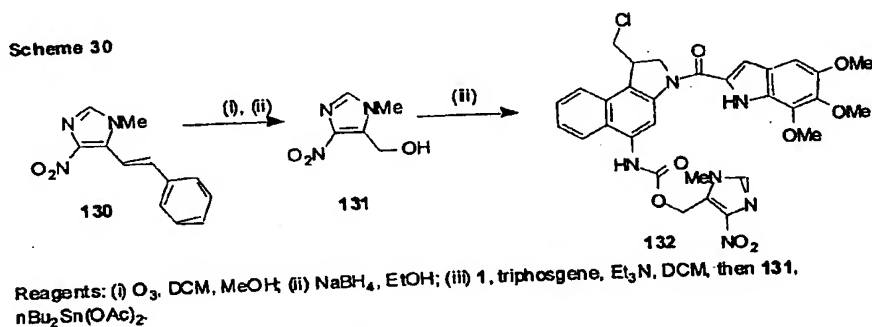


Similarly (Scheme 29), reaction of carbonate 116 with amine 9 using HOBT, molecular sieves, and triethylamine gave the silyl ether 126. Deprotection of silyl ether 126 under acidic conditions gave alcohol 127 which was reacted with 4-nitrophenyl chloroformate to give carbonate 128. Reaction of the carbonate 128 with doxorubicin (13) and triethylamine in DMF gave the carbamate 129.

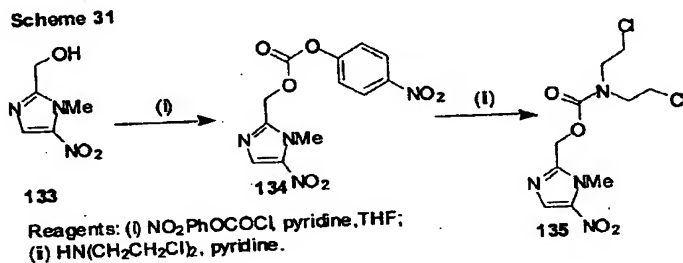


In another example (Scheme 30), ozonolysis of the styrene 130 [D. C. Baker, S. R. Putt, H. D. H. Showalter, *J. Heterocyclic Chem.*, 1983, 30, 629-634.] gave the alcohol 131.

Activation of amine **1** [G. J. Atwell, W. R. Wilson, W. A. Denny, *Bioorg. Med. Chem. Lett.*, 1997, 7, 1483] with triphosgene and triethylamine gave an intermediate isocyanate which was coupled with alcohol **131** using catalytic dibutyltin diacetate to give carbamate **132**.



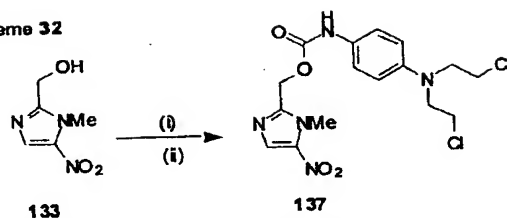
In another example (Scheme 31), treatment of (N-methyl-5-nitro-1H-imidazol-2-yl)methanol (**133**) [C. Rufer, H. J. Kessler, E. Schroder, *J. Med. Chem.* 1971, 14, 94.] with 4-nitrophenylchloroformate gave the carbonate **134**, which was displaced with *N,N*-bis(2-chloroethyl)amine to give the carbamate **135**.



Similarly (Scheme 32), activation of **133** with diphosgene and subsequent reaction with *N,N'*-bis(2-chloroethyl)-1,4-benzenediamine hydrochloride (**136**) gave the carbamate **137**.

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Scheme 32

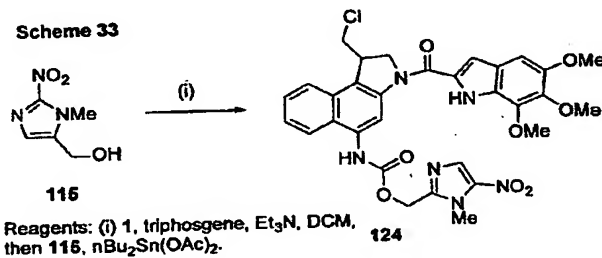


Reagents: (i) diphosgene, Et₃N, THF; (ii) 136, pyridine.

In another example (Scheme 33), activation of amine 1 [G. J. Atwell, W. R. Wilson, W. A. Denny, *Bioorg. Med. Chem. Lett.*, 1997, 7, 1483] with triphosgene and triethylamine gave an intermediate isocyanate which was coupled with alcohol 133 using catalytic dibutyltin

5 diacetate to give carbamate 138.

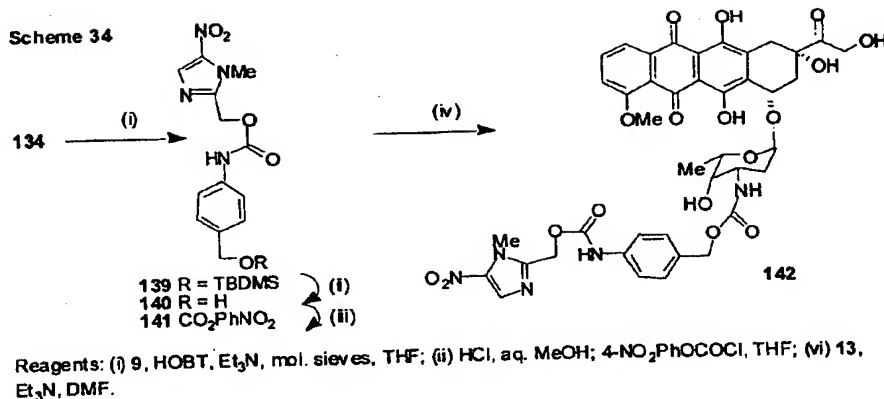
Scheme 33



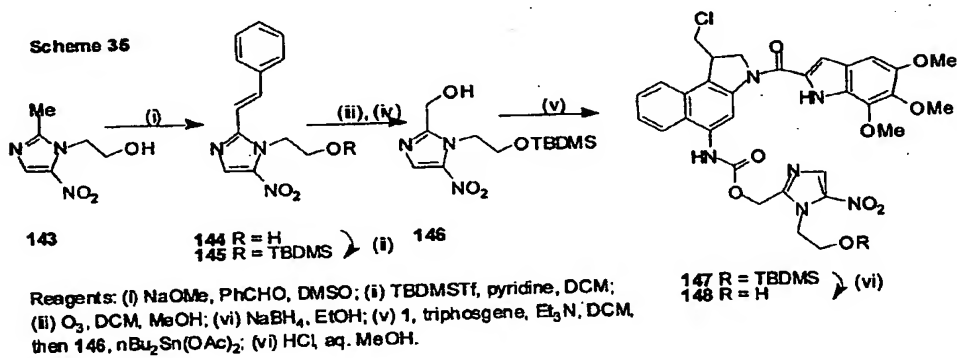
Reagents: (i) 1, triphosgene, Et₃N, DCM, then 115, nBu₂Sn(OAc)₂.

In another example (Scheme 34), reaction of carbonate 134 with amine 9 using HOBT, molecular sieves, and triethylamine gave the silyl ether 139. Deprotection of silyl ether 139 under acidic conditions gave alcohol 140 which was reacted with 4-nitrophenyl chloroformate to carbonate 141. Reaction of the carbonate 141 with doxorubicin (13) and triethylamine in DMF gave the carbamate 142.

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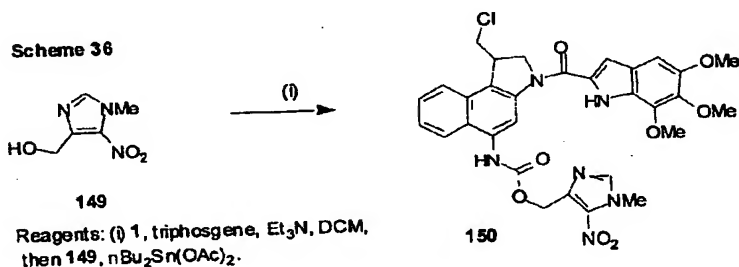


- In another example (Scheme 35), condensation of metronidazole (143) and benzaldehyde gave the styrene 144 which was protected with TBDMS triflate to give 145. Ozonolysis of styrene 145 gave alcohol 146. Activation of amine 1 [G. J. Atwell, W. R. Wilson, W. A. Denny, *Bioorg. Med. Chem. Lett.*, 1997, 7, 1483] with triphosgene and triethylamine gave an intermediate isocyanate which was coupled with alcohol 146 using catalytic dibutyltin diacetate to give carbamate 147. Deprotection under acidic conditions gave the carbamate 148.



Similarly (Scheme 36), activation of amine 1 [G. J. Atwell, W. R. Wilson, W. A. Denny, *Bioorg. Med. Chem. Lett.*, 1997, 7, 1483] with triphosgene and triethylamine gave an intermediate isocyanate which was coupled with alcohol 149 [D. C. Baker, S.R. Putt, H. D. H. Showalter, *J. Heterocyclic Chem.*, 1983, 20, 629-634.] using catalytic dibutyltin diacetate to

5 give carbamate 150.

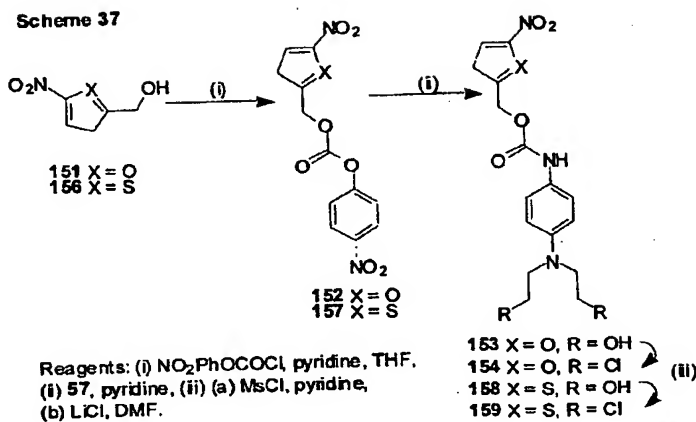


10 In another example (Scheme 37), reaction of the 5-nitrofuran-1-methanol (151) [J. M. Berry, C. Y. Watson, W. J. D. Wish, and M. D. Threadgill, *J. Chem. Soc. Perkin Trans. I*, 1997, 1147.] with 4-nitrophenylchloroformate gave carbonate 152, which was displaced with N^N-bis(2-hydroxyethyl)-1,4-benzenediamine (57) to give the carbamate diol 153. The diol 153 was converted to the dichloride 154 using standard methods.

15 Similarly (Scheme 37), reaction of (5-nitrothien-2-yl)methanol (156) [P. J. Narcombe, R. K. Norris. *Aust. J. Chem.* 1979, 32, 2647] with 4-nitrophenylchloroformate gave carbonate 157, which was displaced with N^N-bis(2-hydroxyethyl)-1,4-benzenediamine (57) to give the carbamate diol 158. The diol 158 was converted to the dichloride 159 using standard methods. The same technique was used on 5-nitrofuran-1-methanol (151) [J.M.

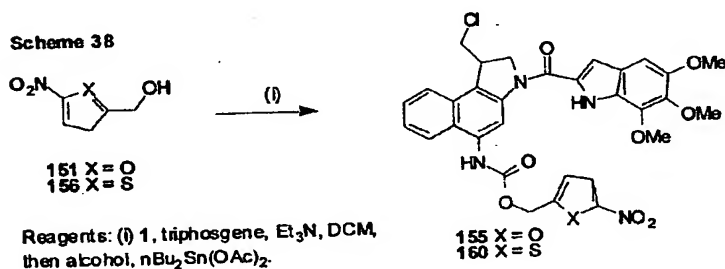
20 Berry, C.Y. Watson, W.J. Wish, and M.D. Threadgill, *J. Chem. Soc. Perkin Trans. I*, 1997, 1147].

- 31 -



- In another example (Scheme 38), activation of amine 1 [G. J. Atwell, W. R. Wilson, W. A. Denny, *Bioorg. Med. Chem. Lett.*, 1997, 7, 1483] with triphosgene and triethylamine gave an intermediate isocyanate which was coupled with alcohol 151 [J. M. Berry, C. Y. Watson, W. J. D. Whish, and M. D. Threadgill, *J. Chem. Soc. Perkin Trans. I*, 1997, 1147.] using catalytic dibutyltin diacetate to give carbamate 155.

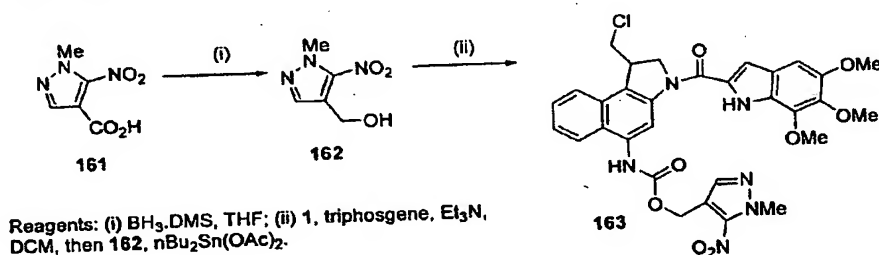
- Similarly (Scheme 38), activation of amine 1 [G. J. Atwell, W. R. Wilson, W. A. Denny, *Bioorg. Med. Chem. Lett.*, 1997, 7, 1483] with triphosgene and triethylamine gave an intermediate isocyanate which was coupled with alcohol 156 [P. J. Narcombe, R. K. Norris, *Aust. J. Chem.* 1979, 32, 2647] using catalytic dibutyltin diacetate to give carbamate 160.



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- In another example (Scheme 39), 1-methyl-5-nitro-1H-pyrazole-4-carboxylic acid (161) [C. C. Cheng, *J. Heterocyclic Chem.* 1968, 5, 195-197] was reduced with borane.dimethyl sulfide to give alcohol 162. Activation of amine 1 [G. J. Atwell, W. R. Wilson, W. A. Denny, *Bioorg. Med. Chem. Lett.*, 1997, 7, 1483] with triphosgene and triethylamine gave an intermediate isocyanate which was coupled with alcohol 162 using catalytic dibutyltin diacetate to give carbamate 163.

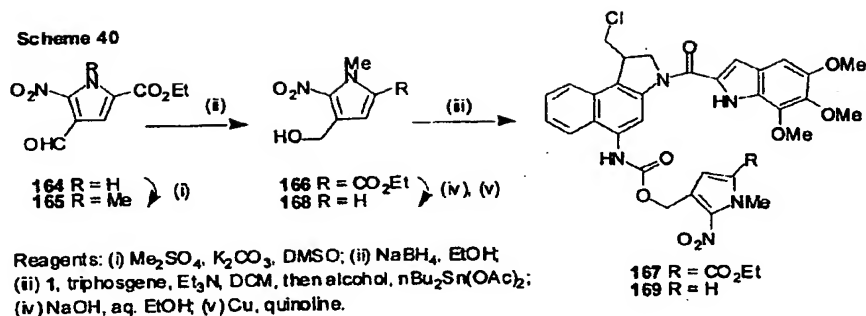
Scheme 39



- In another example (Scheme 40), ethyl 4-formyl-5-nitro-1H-pyrrole-2-carboxylate (164) [P. Fornari, M. Farnier, C. Fournier, *Bull. Soc. Chim. Fr.* 1972, 283-291] was alkylated with dimethyl sulfate to give pyrrole 165. Reduction of 165 with sodium borohydride gave the alcohol 166. Activation of amine 1 [G. J. Atwell, W. R. Wilson, W. A. Denny, *Bioorg. Med. Chem. Lett.*, 1997, 7, 1483] with triphosgene and triethylamine gave an intermediate isocyanate which was coupled with alcohol 166 using catalytic dibutyltin diacetate to give carbamate 167.

- In another example (Scheme 40), hydrolysis of ester 166 followed by decarboxylation with copper in quinoline gave alcohol 168. Activation of amine 1 [G. J. Atwell, W. R. Wilson, W. A. Denny, *Bioorg. Med. Chem. Lett.*, 1997, 7, 1483] with triphosgene and triethylamine gave an intermediate isocyanate which was coupled with alcohol 168 using catalytic dibutyltin diacetate to give carbamate 169.

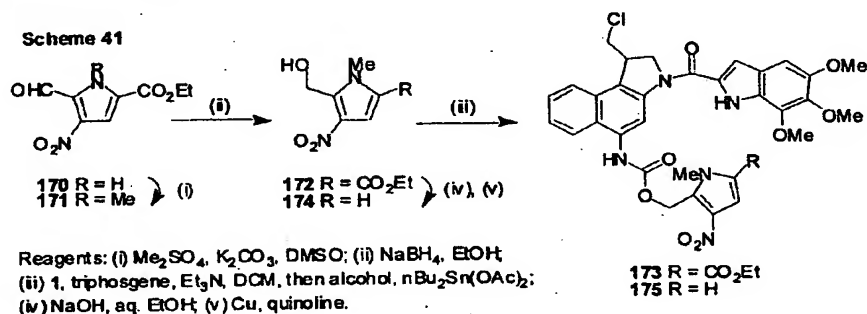
- 33 -



Similarly (Scheme 41), ethyl 5-formyl-4-nitro-1H-pyrrole-2-carboxylate (170) [P. Fornari, M. Farnier, C. Fournier, *Bull. Soc. Chim. Fr.* 1972, 283-291] was alkylated with

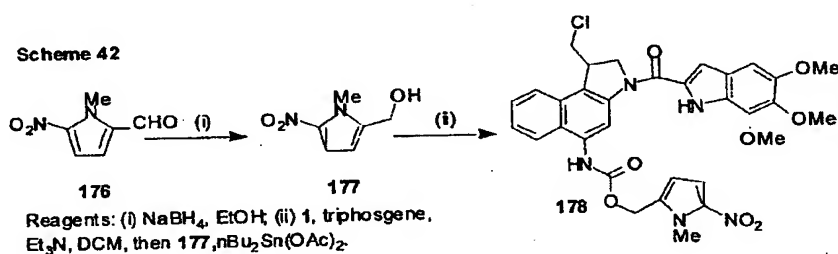
- 5 dimethyl sulfate to give pyrrole 171. Reduction of 171 with sodium borohydride gave the alcohol 172. Activation of amine 1 [G. J. Atwell, W. R. Wilson, W. A. Denny, *Bioorg. Med. Chem. Lett.*, 1997, 7, 1483] with triphosgene and triethylamine gave an intermediate isocyanate which was coupled with alcohol 172 using catalytic dibutyltin diacetate to give carbamate 173.

- 10 In another example (Scheme 41), hydrolysis of ester 172 followed by decarboxylation with copper in quinoline gave alcohol 174. Activation of amine 1 [G. J. Atwell, W. R. Wilson, W. A. Denny, *Bioorg. Med. Chem. Lett.*, 1997, 7, 1483] with triphosgene and triethylamine gave an intermediate isocyanate which was coupled with alcohol 174 using catalytic dibutyltin diacetate to give carbamate 175.



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In another example (Scheme 42), 1-methyl-5-nitro-1*H*-pyrrole-2-carbaldehyde (176) [P. Fournari, *Bull. Soc. Chim. Fr.* 1963, 488-491] was reduced with sodium borohydride to give alcohol 177. Activation of amine 1 [G. J. Atwell, W. R. Wilson, W. A. Denny, *Bioorg. Med. Chem. Lett.*, 1997, 7, 1483] with triphosgene and triethylamine gave an intermediate isocyanate which was coupled with alcohol 177 using catalytic dibutyltin diacetate to give carbamate 178.



In a further preferred aspect, the present invention relates to the use of a compound of formula (I) or (II) as defined in the second aspect of the invention, in conjunction with a nitroreductase enzyme (for example, isolated from *E. coli*) in a method of ADEPT or GDEPT therapy. A drug produced by the action of the nitroreductase enzyme on a compound of formula (I) or (II) may be used for the selective killing of oxic and hypoxic tumour cells in methods of treatment of cancers, for example leukemias and particularly solid cancers including breast, bowel and lung tumours, including small cell lung carcinoma.

The invention also provides a pharmaceutical composition comprising a compound of the formula (I) or (II) as defined in the second aspect of the invention together with a pharmaceutically acceptable carrier or diluent.

Detailed Description of the Invention

GDEPT

20 - Vector systems

In general, the vector for use in GDEPT therapies may be any suitable DNA or RNA vector.

Suitable viral vectors include those which are based upon a retrovirus. Such vectors are widely available in the art. Huber *et al.* (ibid) report the use of amphotropic retroviruses for the transformation of hepatoma, breast, colon or skin cells. Culver *et al.* (Science (1992)

256; 1550-1552) also describe the use of retroviral vectors in GDEPT. Such vectors or vectors derived from them may also be used. Other retroviruses may also be used to make vectors suitable for use in the present invention. Such retroviruses include rous sarcoma virus (RSV).

Englehardt *et al.* (Nature Genetics (1993) 4; 27-34) describe the use of adenovirus based vectors in the delivery of the cystic fibrosis transmembrane conductance product (CFTR) into cells, and such adenovirus based vectors may also be used. Vectors utilising adenovirus promoter and other control sequences may be of use in delivering a system according to the invention to cells in the lung, and hence useful in treating lung tumours.

Other vector systems including vectors based on the Molony murine leukaemia virus are known (Ram, Z *et al.* Cancer Research (1993) 53; 83-88; Dalton & Treisman, Cell (1992) 68; 597-612). These vectors contain the Murine Leukaemia virus (MLV) enhancer cloned upstream at a β -globin minimal promoter. The β -globin 5' untranslated region up to the initiation ATG is supplied to direct efficient translation of the enzyme.

Suitable promoters which may be used in vectors described above, include MLV, CMV, RSV and adenovirus promoters. Preferred adenovirus promoters are the adenovirus early gene promoters. Strong mammalian promoters may also be suitable. An example of such a promoter is the EF-1 α promoter which may be obtained by reference to Mizushima and Nagata ((1990), Nucl. Acids Res. 18; 5322). Variants of such promoters retaining substantially similar transcriptional activities may also be used.

20 - Nitroreductase

Compounds of the formula (I) or (II) can be activated by reduction of one (or more) of the available nitro groups by nitroreductase.

Preferably, the enzyme is a non-mammalian nitroreductase enzyme, such as a bacterial nitroreductase. An *E.coli* nitroreductase as disclosed in WO93/08288 is particularly preferred.

The enzyme may be modified by standard recombinant DNA techniques, e.g. by cloning the enzyme, determining its gene sequence and altering the gene sequence by methods such as truncation, substitution, deletion or insertion of sequences for example by site-directed mutagenesis. Reference may be made to "Molecular Cloning" by Sambrook *et al.* (1989, Cold Spring Harbor) for discussion of standard recombinant DNA techniques. The modification made may be any which still leaves the enzyme with the ability to reduce the nitro group in formula I or II but alters other properties of the enzyme, for example its rate of reaction or selectivity.

In addition, small truncations in the N- and/or C-terminal sequence may occur as a result of the manipulations required to produce a vector in which a nucleic acid sequence encoding the enzyme is linked to the various other vector sequences.

ADEPT

- 5 For applications in ADEPT systems, an antibody directed against a tumour specific marker is linked to the nitroreductase enzyme, which may be modified as described above. The antibody may be monoclonal or polyclonal. For the purposes of the present invention, the term "antibody", unless specified to the contrary, includes fragments of whole antibodies which retain their binding activity for a tumour target antigen. Such fragments include Fv, 10 F(ab') and F(ab')₂ fragments, as well as single chain antibodies. Furthermore, the antibodies and fragments thereof may be humanised antibodies, e.g. as described in EP-A-239400.

- The antibodies may be produced by conventional hybridoma techniques or, in the case of modified antibodies or fragments, by recombinant DNA technology, eg by the expression in a suitable host vector of a DNA construct encoding the modified antibody or fragment 15 operably linked to a promoter. Suitable host cells include bacterial (eg. *E.coli*), yeast, insect and mammalian. When the antibody is produced by such recombinant techniques the enzyme may be produced by linking a nucleic acid sequence encoding the enzyme (optionally modified as described above) to the 3' or 5' end of the sequence of the construct encoding the antibody or fragment thereof.

20 Applications of the invention

- Compounds of the invention can be used *in vitro* or *in vivo* for a range of applications. For example, a number of vector systems for the expression of nitroreductase in a cell have been developed. The further development of such systems (e.g. the development of promoters suitable for specific cell types) requires suitable candidate prodrugs capable of killing cells 25 when activated by nitroreductase. Prodrug compounds of the present invention may be used in such model systems. The model systems may be *in vitro* model systems or xenograft model systems comprising for example human tumour cells implanted in nude mice.

- Compounds of the invention which are not activatable by an enzyme may be tested *in vitro* against panels of different tumour cells types to determine efficacy against such tumour 30 cells. The efficacy of compounds of the invention against a range of tumour cell types may be used as points of reference for the development of further antitumour compounds. Compounds of the present invention may also be tested in combination with additional anti-

cancer compounds to determine potential combination drug systems, for example combinations which are synergistic.

The compounds of the invention may also be used in a method of treatment of the human or animal body. Such treatment includes a method of treating the growth of neoplastic cells in a patient with neoplastic disease which comprises administering to a patient in need of treatment a compound of formula (I) or (II) of the invention as part of an ADEPT or GDEPT therapy system. Neoplastic diseases include leukaemia and solid tumours such as breast, bowel and lung tumours including small cell lung carcinoma.

It will be understood that where treatment of tumours is concerned, treatment includes any measure taken by the physician to alleviate the effect of the tumour on a patient. Thus, although complete remission of the tumour is a desirable goal, effective treatment will also include any measures capable of achieving partial remission of the tumour as well as a slowing down in the rate of growth of a tumour including metastases. Such measures can be effective in prolonging and/or enhancing the quality of life and relieving the symptoms of the disease.

15 ADEPT therapy

The antibody/enzyme conjugate for ADEPT can be administered simultaneously but it is often found preferable, in clinical practice, to administer the enzyme/agent conjugate before the prodrug, e.g. up to 72 hours or even 1 week before, in order to give the enzyme/agent conjugate an opportunity to localise in the region of the tumour target. By operating in this way, when the prodrug is administered, conversion of the prodrug to the cytotoxic agent tends to be confined to the regions where the enzyme/agent conjugate is localised, i.e. the region of the target tumour, and the premature release of the compound produced by the action of the nitroreductase on the compound of formula (I) or (II) is minimised.

In ADEPT the degree of localisation of the enzyme/agent conjugate (in terms of the ratio of localized to freely circulating active conjugate) can be further enhanced using the clearance and/or inactivation systems described in WO89/10140. This involves, usually following administration of the conjugate and before administration of the prodrug, the administration of a component (a "second component") which is able to bind to part of the conjugate so as to inactivate the enzyme and/or accelerate the clearance of the conjugate from the blood. Such a component may include an antibody to the enzyme component of the system which is capable of inactivating the enzyme.

The second component may be linked to a macromolecule such as dextran, a liposome,

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albumin, macroglobulin or a blood group O erythrocyte so that the second component is restrained from leaving the vascular compartment. In addition or as an alternative, the second component may include a sufficient number of covalently bound galactose residues, or residues of other sugars such as lactose or mannose, so that it can bind the conjugate in plasma but be removed together with the conjugate from plasma by receptors for galactose or other sugars in the liver. The second component should be administered and designed for use such that it will not, to any appreciable extent, enter the extravascular space of the tumour where it could inactivate localised conjugate prior to and during administration of the prodrug.

In ADEPT systems, the dose of the prodrug and conjugate will ultimately be at the discretion of the physician, who will take into account such factors as the age, weight and condition of the patient. Suitable doses of prodrug and conjugate are given in Bagshawe *et al.* Antibody, Immunoconjugates, and Radiopharmaceuticals (1991), 4, 915-922. A suitable dose of conjugate may be from 500 to 200,000 enzyme units/m² (e.g. 20,000 enzyme units/m²) and a suitable dose of prodrug may be from about 0.1 to 200 mg/Kg, preferably about from 10 to 100 mg/Kg per patient per day.

In order to secure maximum concentration of the conjugate at the site of desired treatment, it is normally desirable to space apart administration of the two components by at least 4 hours. The exact regime will be influenced by various factors including the nature of the tumour to be targeted and the nature of the prodrug, but usually there will be an adequate concentration of the conjugate at the site of desired treatment within 48 hours.

The ADEPT system when used with nitroreductase also preferably comprises a suitable cofactor for the enzyme. Suitable cofactors include a riboside or ribotide of nicotinic acid or nicotinamide.

The antibody/enzyme conjugate may be administered by any suitable route usually used in ADEPT therapy. This includes parenteral administration of the antibody in a manner and in formulations similar to that described below.

GDEPT therapy

For use of the vectors in therapy, the vectors will usually be packaged into viral particles and the particles delivered to the site of the tumour, as described in for example Ram *et al.* (ibid). The viral particles may be modified to include an antibody, fragment thereof (including a single chain) or tumour-directed ligand to enhance targeting of the tumour. Alternatively the vectors may be packaged into liposomes. The liposomes may be targeted to

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a particular tumour. This can be achieved by attaching a tumour-directed antibody to the liposome. Viral particles may also be incorporated into liposomes. The particles may be delivered to the tumour by any suitable means at the disposal of the physician. Preferably, the viral particles will be capable of selectively infecting the tumour cells. By "selectively infecting" it is meant that the viral particles will primarily infect tumour cells and that the proportion of non-tumour cells infected is such that the damage to non-tumour cells by administration of a prodrug will be acceptably low, given the nature of the disease being treated. Ultimately, this will be determined by the physician.

One suitable route of administration is by injection of the particles in a sterile solution. Viruses, for example isolated from packaging cell lines may also be administered by regional perfusion or direct intratumoral direction, or direct injection into a body cavity (intracavitary administration), for example by intra-peritoneum injection.

The exact dosage regime for GDEPT will, of course, need to be determined by individual clinicians for individual patients and this, in turn, will be controlled by the exact nature of the prodrug and the cytotoxic agent to be released from the prodrug but some general guidance can be given. Chemotherapy of this type will normally involve parenteral administration of modified virus and administration by the intravenous route is frequently found to be the most practical.

In GDEPT systems the amount of virus or other vector delivered will be such as to provide a similar cellular concentration of enzyme as in the ADEPT system mentioned above. Typically, the vector will be administered to the patient and then the uptake of the vector by transfected or infected (in the case of viral vectors) cells monitored, for example by recovery and analysis of a biopsy sample of targeted tissue. This may be determined by clinical trials which involve administering a range of trial doses to a patient and measuring the degree of infection or transfection of a target cell or tumour. The amount of prodrug required will be similar to or greater than that for ADEPT systems.

In using a GDEPT system the prodrug will usually be administered following administration of the vector encoding an enzyme. Suitable doses of prodrug are from about 0.1 to 200 mg/Kg, preferably about from 10 to 100 mg/Kg per patient per day.

30 Administration of prodrug

While it is possible for a compound of formula (I) or (II) to be administered alone it is preferable to present it as a pharmaceutical formulation. Suitable formulations comprise the

compounds, together with one or more acceptable carriers thereof and optionally other therapeutic ingredients. The carrier or carriers must be "acceptable" in the sense of being compatible with the other ingredients of the formulation and not deleterious to the recipients thereof, for example, liposomes. Suitable liposomes include, for example, those comprising the positively charged lipid (N[1-(2,3-dioleoyloxy)propyl]-N,N,N-triethylammonium (DOTMA), those comprising dioleoylphosphatidylethanolamine (DOPE), and those comprising 3 β [N-(N',N'-dimethylaminoethane)-carbamoyl]cholesterol (DC-Chol).

Formulations suitable for parenteral or intramuscular administration include aqueous and non-aqueous sterile injection solutions which may contain anti-oxidants, buffers, bacteriostats, bactericidal antibiotics and solutes which render the formulation isotonic with the blood of the intended recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening agents, and liposomes or other microparticulate systems which are designed to target the compound to blood components or one or more organs. The formulations may be presented in unit-dose or multi-dose containers, for example sealed ampoules and vials, and may be stored in a freeze-dried (lyophilized) condition requiring only the addition of the sterile liquid carrier, for example water, for injections, immediately prior to use. Injection solutions and suspensions may be prepared extemporaneously from sterile powders, granules and tablets of the kind previously described.

It should be understood that in addition to the ingredients particularly mentioned above the formulations may include other agents conventional in the art having regard to the type of formulation in question. Of the possible formulations, sterile pyrogen-free aqueous and non-aqueous solutions are preferred.

The doses may be administered sequentially, eg. at daily, weekly or monthly intervals, or in response to a specific need of a patient. Preferred routes of administration are oral delivery and injection, typically parenteral or intramuscular injection or intratumoral injection.

The exact dosage regime will, of course, need to be determined by individual clinicians for individual patients and this, in turn, will be controlled by the exact nature of the compound of formula (I) or (II) but some general guidance can be given. Typical dosage ranges generally will be those described above which may be administered in single or multiple doses. Other doses may be used according to the condition of the patient and other factors at the discretion of the physician.

The following Examples illustrate the invention.

General procedures

Analyses were carried out in the Microchemical Laboratory, University of Otago, Dunedin,

- 5 NZ. Melting points were determined on an Electrothermal 2300 Melting Point Apparatus. IR spectra were recorded on a Midac FT-IR as KBr discs, unless otherwise stated. NMR spectra were obtained on a Bruker AM-400 spectrometer at 400 MHz for ^1H and 100 MHz for ^{13}C spectra. Spectra were obtained in CDCl_3 unless otherwise specified, and are referenced to Me_4Si . Chemical shifts and coupling constants were recorded in units of ppm and Hz,
- 10 respectively. Assignments were determined by APT, COSY, HSQC, and HMBC experiments. Mass spectra were determined on a VG-70SE mass spectrometer using an ionizing potential of 70 eV at a nominal resolution of 1000. High resolution spectra were obtained at nominal resolutions of 3000, 5000, or 10000 as appropriate. All spectra were obtained as electron impact (EI) using PFK as the reference unless otherwise stated. Solutions
- 15 in organic solvents were dried with anhydrous Na_2SO_4 . Solvents were evaporated under reduced pressure on a Buchi rotary evaporator. Thin-layer chromatography was carried out on aluminium-backed silica gel plates (Merck 60 F_{254}) with visualisation of components by UV light (254 nm) or exposure to I_2 . Column chromatography was carried out on silica gel, (Merck 230-400 mesh). All compounds designated for biological testing were analyzed at
- 20 >99% purity by reverse phase HPLC using a Philips PU4100 liquid chromatograph, a Phenomenex BondClone 10-C18 stainless steel column (300mm \times 3.9 mm i.d.) and a Philips PU4120 diode array detector. Chromatograms were run using various gradients of aqueous (1 M NaH_2PO_4 , 0.75 M heptanesulfonic acid, 0.5 M dibutylammonium phosphate, and MilliQ water in a 1:1:1:97 ratio) and organic (80% MeOH/MilliQ water) phases. DCM refers to
- 25 dichloromethane; DIEA refers to diisopropylethylamine, DMF refers to dry dimethyl formamide; DMSO refers to dimethylsulphoxide; ether refers to diethyl ether; EtOAc refers to ethyl acetate; EtOH refers to ethanol; iPr_2O refers to diisopropyl ether; light petroleum refers to petroleum ether, boiling range 40-60 $^\circ\text{C}$; MeOH refers to methanol; THF refers to tetrahydrofuran dried over sodium benzophenone ketyl. All solvents were freshly distilled.

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Example 1. Preparation of 4-nitrobenzyl 4-(1,3-benzothiazol-2-yl)phenylcarbamate (8). 4-Nitrophenyl chloroformate (0.15 g, 0.46 mmol) was added to a stirred solution of 2-

(4-aminophenyl)benzthiazole (7) [D-F. Shi, T.D. Bradshaw, S. Wrigley, C.J. McCall, P. Lelieveld, I. Fitchner, M.F.G. Stevens. *J. Med. Chem.*, 1996, 39, 3375] in pyridine (5 mL) and the solution stirred at 20 °C for 2 h. The solution was dilute with water (10 mL) and the mixture stirred for 40 min, filtered and the solid triturated with hot EtOH to give 8 (157 mg, 87%) as a pale green powder, mp 232-234 °C; ¹H NMR [(CD₃)₂SO]δ 10.31 (s, 1 H, OCONH), 8.29 (ddd, *J* = 8.7, 3.2, 2.2 Hz, 2 H, H 3", H 5"), 8.11 (d, *J* = 8.3 Hz, 1 H, H 4), 8.04 (br d, *J* = 8.7 Hz, 2 H, H 2", H 6"), 8.02 (d, *J* = 8.3 Hz, 1 H, H 7), 7.72 (br d, *J* = 8.7 Hz, 2 H, H 2', H 6'), 7.68 (br d, *J* = Hz, 2 H, H 3', H 5'), 7.51-7.55 (m, 1 H, H 5), 7.40-7.46 (m, 1 H, H 6), 5.32 (s, 2 H, CH₂O); Anal. (C₂₁H₁₅N₃O₄S) C, H, N.

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Example 2A. Preparation of 2-methoxy-4-nitrobenzyl 4-[bis(2-chloroethyl)amino]phenylcarbamate (32).

Methyl 2-methoxy-4-nitrobenzoate (28). An ethereal solution of diazomethane (CAUTION) was added to a solution of 4-nitrosalicylic acid (27) (1.0 g, 5.46 mmol) in ether (50 mL) until a yellow colour persisted and the solution stood at 20 °C for 4 h. The reaction was quenched with glacial acetic acid (2 mL), poured into sat. aq. NaHCO₃ solution and extracted with ether (2 × 50 mL). The combined organic fractions were dried and the solvent evaporated to give 28 (1.11 g, 96%) as white needles, mp (ether) 89-90 °C; IR N 1740, 1526, 1252, and 1086 cm⁻¹; ¹H NMR δ 7.89 (d, *J* = 8.3 Hz 1 H, H 5), 7.82-7.85 (m, 2 H, H 3, H 6), 4.01 (s, 3 H, OCH₃), and 3.94 (s, 3 H, OCH₃); ¹³C NMR 165.2 (CO₂), 159.2 (C 2), 150.7 (C 4), 132.0 (C 1), 126.0 (C 6), 115.0 (C 5), 106.9 (C 3), 56.6 (OCH₃), and 52.6 (OCH₃); Anal. (C₉H₇NO₅) C, H, N.

2-Methoxy-4-nitrobenzyl alcohol (29). A solution of 28 (0.9 g, 4.26 mmol) in THF (20 mL) was added dropwise to a stirred solution of DIBALH (1 M solution in toluene, 13.4 mL, 13.4 mmol) in THF (20 mL) at 2 °C and the solution stirred at 2 °C for 15 min. The solvent was evaporated and residue partitioned between EtOAc (100 mL) and water (100 mL). The aqueous fraction was extracted with EtOAc (2 × 50 mL) and the combined organic fraction dried and the solvent evaporated. The residue was purified by chromatography, eluting with 50% EtOAc/light petroleum, to give 29 (0.74 g, 93%) as cream needles, mp (EtOAc/light petroleum) 103-104 °C; IR n 3310, 1523, 1250, and 1036 cm⁻¹; ¹H NMR δ 7.86 (dd, *J* = 8.3, 2.1 Hz, 1 H, H 5), 7.71 (d, *J* = 2.1 Hz, 1 H, H 3), 7.52 (d,

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$J = 8.3$ Hz, 1 H, H 6), 4.76 (d, $J = 5.5$ Hz, 2 H, CH_2O), 3.96 (s, 3 H, OCH_3), and 2.27 (br s, 1 H, OH); ^{13}C NMR δ 157.1, 148.3, 136.6, 127.9, 116.0, 105.0, 60.7, and 55.9; Anal. ($\text{C}_8\text{H}_9\text{NO}_4$) C, H, N.

- 5 **2-Methoxy-4-nitrobenzyl 4-[bis(2-hydroxyethyl)amino]phenylcarbamate (31).** Pyridine (91 μL , 1.13 mmol) was added dropwise to a stirred solution of 29 (207 mg, 1.13 mmol) and triphosgene (117 mg, 0.40 mmol) in THF (10 mL) at 5 °C and the suspension stirred at 5 °C for 1 h. A solution of N,N' -bis(2-hydroxyethyl)-1,4-benzenediamine (57) [prepared by catalytic hydrogenation of N,N -bis-(2-hydroxyethyl) 4-nitroaniline (30)] (244 mg, 1.24 mmol) with Pd/C under H_2 (60 psi) in EtOH) in THF (10 mL) and DMF (10 mL) was added and the mixture stirred at 20 °C for 16 h. The solvent was evaporated and the residue purified by chromatography, eluting with EtOAc to give 31 (251 mg, 55%) as orange prisms, mp (EtOAc) 153-154 °C; ^1H NMR δ 9.45 (br s, 1 H, OCONH), 7.90 (dd, $J = 8.3$, 2.0 Hz, 1 H, H 5'), 7.80 (d, $J = 2.0$ Hz, 1 H, H 3'), 7.60 (br d, $J = 8.3$ Hz, 1 H, H 6'), 7.21 (br d, $J = 9.0$ Hz, 2 H, H 2, H 6), 6.61 (d, $J = 9.0$ Hz, 2 H, H 3, H 5), 5.17 (s, 2 H, CH_2O), 4.71 (t, $J = 5.4$ Hz, 2 H, 2 \times OH), 3.97 (s, 3 H, OCH_3), 3.48-3.53 (m, 4 H, 2 \times CH_2O), 3.32-3.37 (m, 4 H, 2 \times CH_2N); ^{13}C NMR δ 157.4, 153.7, 148.6, 144.6, 133.2, 129.0, 127.8, 120.7 (2), 116.0, 111.9 (2), 105.9, 60.6, 58.7 (2), 56.7, 53.9 (2); Anal. ($\text{C}_{19}\text{H}_{23}\text{N}_3\text{O}_7$) C, H, N.
- 20 **2-Methoxy-4-nitrobenzyl 4-[bis(2-chloroethyl)amino]phenylcarbamate (32).** Methanesulphonyl chloride (129 μL , 1.67 mmol) was added dropwise to a stirred solution of 31 (226 mg, 0.55 mmol) in pyridine (10 mL) at 20 °C and the solution stirred for 1 h. The solvent was evaporated and the residue partitioned between DCM/water (100 mL). The aqueous fraction was extracted with DCM (2 \times 50 mL) and the combined organic fraction washed with brine (50 mL), dried and the solvent evaporated. The residue was dissolved in DMF (10 mL), LiCl (0.15 g, 3.34 mmol) added, and the mixture stirred at 80 °C for 2 h. The solvent was evaporated and the residue partitioned between EtOAc/water (100 mL). The aqueous fraction was extracted with EtOAc (2 \times 25 mL). The combined organic fraction was washed with brine (30 mL), dried, and the solvent evaporated. The residue was purified by chromatography, eluting with 25% EtOAc/light petroleum, to give 32 (230 mg, 93%) as pale green needles, mp (EtOAc/light petroleum) 129-130 °C; ^1H NMR δ 7.84 (dd, $J = 8.3$, 2.1 Hz, 1 H, H 5'), 7.72 (d, $J = 2.1$ Hz, 1 H, H 3'), 7.51 (d, $J = 8.3$ Hz, 1 H, H
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6'), 7.27 (br d, $J = 9.0$ Hz, 2 H, H 2, H 6), 6.65 (ddd, $J = 9.0, 3.5, 2.1$ Hz, 2 H, H 3, H 5), 5.29, (s, 2 H, CH_2O), 3.96 (s, 3 H, OCH_3), 3.68-3.72 (m, 4 H, $2 \times \text{CH}_2\text{N}$), 3.58-3.63 (m, 4 H, $2 \times \text{CH}_2\text{Cl}$); ^{13}C NMR δ 157.2, 153.4, 148.6, 142.9, 132.2, 128.5, 128.1, 121.4 (2), 115.7, 112.7 (2), 105.3, 61.3, 56.0, 53.7 (2), 40.5 (2); Anal. ($\text{C}_{19}\text{H}_{21}\text{Cl}_2\text{N}_3\text{O}_5$) C, H, N.

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Example 2B. Preparation of 2-methoxy-4-nitrobenzyl 3-(chloromethyl)-1-[(5,6,7-trimethoxy-1*H*-indol-2-yl)carbonyl]-2,3-dihydro-1*H*-indol-6-ylcarbamate (34).

- Pyridine (20 μL , 0.25 mmol) was added dropwise to a stirred solution of 2-methoxy-4-nitrobenzyl alcohol (29) (45 mg, 0.25 mmol) and triphosgene (26 mg, 0.09 mmol) in THF (10 mL) at 5 °C and the suspension stirred at 5 °C for 1 h. A solution of 6-amino-3-(chloromethyl)-1-[(5,6,7-trimethoxyindol-2-yl)carbonyl]indoline (33) [M. Tercel and W. A. Denny, *J. Chem. Soc. Perkin Trans. 1*, 1998, 509] (102 mg, 0.25 mmol) in THF (10 mL) was added and the mixture stirred at 20 °C for 16 h. The suspension was filtered and the solvent evaporated. The residue was purified by chromatography, eluting with
- 15 40%EtOAc/DCM, to give 34 (102 mg, 65%) as a tan powder, mp (DCM/pet. ether) 144-150 °C; ^1H NMR δ 9.74 (s, 1 H, indole-NH), 8.26 (d, $J = 0.8$ Hz, 1 H, H 7), 7.62-7.68 (m, 3 H, H 5, H 3'', H 5''), 7.58 (br s, 1 H, OCONH), 7.35 (br d, $J = 8.1$ Hz, 1 H, H 6''), 7.20 (d, $J = 8.3$ Hz, 1 H, H 4'), 6.91 (d, $J = 2.1$ Hz, 1 H, H 3'), 6.83 (s, 1 H, H 4'), 5.21 (s, 2 H, CH_2O), 4.58 (dd, $J = 10.6, 8.9$ Hz, 1 H, H 2), 4.41 (d, $J = 10.6, 4.3$ Hz, 1 H, H 2), 4.03, (s, 20 H, OCH_3), 3.92 (s, 3 H, OCH_3), 3.90 (s, 3 H, OCH_3), 3.89 (s, 3 H, OCH_3), 3.76-3.80 (m, 2 H, H 3, CH_2Cl), 3.51 (dd, $J = 11.7, 10.6$ Hz, 1 H, CH_2Cl); ^{13}C NMR δ 160.5, 157.1, 153.1, 150.2, 148.4, 144.0, 140.5, 138.8, 138.7, 132.0, 129.5, 128.3, 126.0, 125.6, 124.5, 123.5, 115.6, 115.5, 108.8, 106.7, 105.5, 97.5, 61.4, 61.3, 61.1, 56.2, 55.9, 54.8, 46.9, 43.2; MS (FAB⁺) m/z 627 (MH^+ , 4%), 625 (MH^+ , 12), 234 (25), 149 (100); HRMS (FAB⁺) calc. for
- 25 $\text{C}_{30}\text{H}_{30}^{35}\text{ClN}_4\text{O}_{10}$ (MH^+) m/z 625.1701, found 625.1690; $\text{C}_{30}\text{H}_{30}^{37}\text{ClN}_4\text{O}_{10}$ (MH^+) m/z 627.1672, found 627.1623; Anal. ($\text{C}_{30}\text{H}_{29}\text{ClN}_4\text{O}_9$) C, H, N.

Example 2C. Preparation of 2-methoxy-4-nitrobenzyl 1-(chloromethyl)-3-[(5,6,7-trimethoxy-1*H*-indol-2-yl)carbonyl]-2,3-dihydro-1*H*-benzo[e]indol-5-ylcarbamate

- 30 (35). Phosgene (300 μL , 0.3 mmol, 1M in toluene) was added to a stirred solution of 2-methoxy-4-nitrobenzyl alcohol (29) (20 mg, 0.11 mmol) in THF (10 mL) and stirred at 20

°C for 16 h. The solvent was evaporated, the residue dissolved in THF (10 mL), a solution of amine 1 [G. J. Atwell, W. R. Wilson, W. A. Denny, *Bioorg. Med. Chem. Lett.*, 1997, 7, 1483] (50 mg, 0.11 mmol) in THF (10 mL) was added and the solution stirred at 20 °C for 4 days. The solvent was evaporated and the residue purified by chromatography, eluting with 50% EtOAc/light petroleum to give 35 (31 mg, 43%) as a tan solid, mp (EtOAc/light petroleum) 162-165 °C; ¹H NMR δ 9.52 (s, 1 H, indole-NH), 8.90 (s, 1 H, OCONH), 7.90 (d, *J* = 8.7 Hz, 1 H, H 6), 7.80 (d, *J* = 8.7 Hz, 1 H, H 5"), 7.77 (d, *J* = 8.4 Hz, 1 H, H 9), 7.70 (br s, 1 H, H 3"), 7.50-7.57 (m, 2 H, H 8, H 6"), 7.42-7.47 (m, 1 H, H 7), 7.25 (br s, 1 H, H 4), 6.99 (d, *J* = 2.2 Hz, 1 H, H 3'), 6.87 (s, 1 H, H 4'), 5.34 (d, *J* = 1.9 Hz, 2 H, CH₂O), 4.78 (dd, *J* = 10.7, 1.6 Hz, 1 H, H 2), 4.64 (dd, *J* = 10.7, 8.8 Hz, 1 H, H 2), 4.07-4.17 (m, 5 H, H 1, CH₂Cl, OCH₃), 3.95 (s, 3 H, OCH₃), 3.94 (s, 3 H, OCH₃), 3.91 (s, 3 H, OCH₃), 3.45 (t, *J* = 10.9 Hz, 1 H, CH₂Cl); ¹³C NMR δ 160.3, 157.2, 154.0, 150.2, 148.6, 141.6, 140.6, 138.9, 133.9, 132.0, 129.7, 129.6, 128.8, 127.4, 127.2, 125.6, 125.4, 125.0, 123.6, 123.1, 123.0, 121.8, 122.4, 115.7, 106.5, 105.1, 97.6, 61.8, 61.5, 61.1, 56.2, 56.0, 54.9, 45.8, 43.4; MS (FAB⁺) *m/z* 675 (MH⁺, 10%), 677 (4), 659 (1), 639 (1), 517 (5), 234 (25); HRMS (FAB⁺) calc. for C₃₅H₃₂³⁵CIN₄O₉ (MH⁺) *m/z* 675.1858, found 674.1832; calc for C₃₅H₃₂³⁷CIN₄O₉ (MH⁺) *m/z* 677.1828, found 677.1834; Anal. (C₃₄H₃₁CIN₄O₉·H₂O) C, H, N.

20 Example 2D. Preparation of 2-methoxy-4-nitrobenzyl 4-(1,3-benzothiazol-2-yl)phenylcarbamate (36). Pyridine (36 mL, 0.44 mmol) was added dropwise to a stirred solution of alcohol of 2-methoxy-4-nitrobenzyl alcohol (29) (81 mg, 0.44 mmol) and triphosgene (66 mg, 0.22 mg) in DCM (10 mL) and the mixture was stirred at 20 °C for 20 min. A solution of 2-(4-aminophenyl)benzthiazole (7) [D-F. Shi, T. D. Bradshaw, S. Wrigley, C. J. McCall, P. Lelieveld, I. Fitchner, M. F. G. Stevens. *J. Med. Chem.*, 1996, 39, 3375] (100 mg, 0.44 mmol) in DCM (5 mL) and the mixture stirred at 20 °C for 4 h. The mixture was partitioned between EtOAc (100 mL) and sat. aq. KHCO₃ solution (50 mL), the organic fraction dried and the solvent evaporated. The residue was slurried in warm EtOAc/MeOH (1:1, 20 mL), filtered and the solvent evaporated to give 36 (123 mg, 64%) as a pale green powder mp (EtOH) 213-214 °C; ¹H NMR [(CD₃)₂SO] δ 10.31 (s, 1 H, OCONH), 8.11 (d, *J* = 7.8 Hz, 1 H, H 4), 8.01-8.06 (m, 3 H, H 7, H 2', H 6'), 7.92 (dd, *J* = 8.3, 2.2 Hz, 1 H, H 5"), 7.81 (d, *J* = 2.2 Hz, 1 H, H 3"), 7.65-7.69 (m, 3 H, H 3', H 5' H 6"),

7.51-7.55 (m, 1 H, H 5), 7.40-7.46 (m, 1 H, H 6), 5.27 (s, 2 H, CH₂O) 3.98 (s, 3 H, OCH₃); ¹³C NMR [(CD₃)₂SO] δ 166.9, 157.1, 153.6, 152.9, 148.3, 141.8, 134.2, 132.0, 129.0 (2), 128.1, 127.0, 126.5, 125.1, 122.4, 122.1, 118.3 (2), 115.5, 105.5, 60.7, 56.2; Anal. (C₂₂H₁₇N₃O₅S) C, H, N.

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Example 2E. Preparation of 2-methoxy-4-nitrobenzyl doxorubicin carbamate (38).

2-Methoxy-4-nitrobenzyl 4-nitrophenyl carbonate (37). A solution of 4-nitrophenyl chloroformate (1.00 g, 4.97 mmol) in pyridine (4 mL) was added dropwise to a stirred solution of 2-methoxy-4-nitrobenzyl alcohol (29) (617 mg, 3.31 mmol) in pyridine (15 mL) at 20 °C and the solution stirred for 16 h. The solvent was evaporated and the residue purified by chromatography, eluting with a gradient (20-50%) EtOAc/light petroleum, to give 37 (928 mg, 80%) as pale yellow solid, mp (EtOAc/light petroleum) 105-106 °C; ¹H NMR δ 8.28 (ddd, *J* = 9.2, 3.1, 2.1 Hz, 2 H, H 3'), 7.89 (dd, *J* = 8.3, 2.1 Hz, 1 H, H 5), 7.77 (d, *J* = 2.1 Hz, 1 H, H 3), 7.58 (d, *J* = 8.3 Hz, 1 H, H 6), 7.40 (ddd, *J* = 8.3, 3.1, 2.1 Hz, 2 H, H 2'), 5.41 (s, 2 H, CH₂O), 4.00 (s, 3 H, OCH₃); ¹³C NMR δ 157.6 (C 2), 155.4 (OCO₂), 152.3 (C 1), 149.2 (C 4), 145.5 (C 4'), 129.8 (C 1), 129.3 (C 6), 125.3 (C 2'), 121.7 (C 3'), 115.8 (C 5), 105.5 (C 3), 65.3 (CH₂O), 56.2 (OCH₃); Anal. (C₁₅H₁₂N₂O₈) C, H, N.

2-Methoxy-4-nitrobenzyl doxorubicin carbamate (38). A solution of carbonate 37 (23 mg, 66 μmol) in DMF (2 mL) was added to a solution of doxorubicin (13) (30 mg, 55 μmol) and Et₃N (9 mL 66 μmol) in DMF (5 mL) at 20 °C and the solution stirred for 16 h. The solvent was evaporated and the residue purified by chromatography, eluting with a gradient (0-5%) of MeOH/DCM, to give 38 (37 mg, 88%) as a red solid, mp (DCM) 159-161 °C; ¹H NMR δ 13.97 (s, 1 H, 6-OH), 13.22 (s, 1 H, 11-OH), 8.02 (dd, *J* = 8.0, 1.0 Hz, 1 H, H 1), 7.77-7.81 (m, 2 H, H 2, H 5'), 7.66 (br s, 1 H, H 3'), 7.41 (d, *J* = 8.0 Hz, 1 H, H 6'), 7.39 (dd, *J* = 8.0, 1.0 Hz, 1 H, H 3), 5.52 (br d, *J* = 3.3 Hz, 1 H, H 1'), 5.29 (br s, 1 H, H 7), 5.25 (d, *J* = 8.7 Hz, 1 H, OCONH), 5.13 (2 d, *J* = 14.0 Hz, 2 H, CH₂O), 4.75 (s, 2 H, H 14), 4.51 (s, 1 H, 9-OH), 4.13-4.17 (m, 1 H, H 5'), 4.08 (s, 3 H, 4-OCH₃), 3.90 (s, 3 H, 2'-OCH₃), 3.84-3.88 (m, 1 H, H 3'), 3.69 (s, 1 H, H 4'), 3.24 (dd, *J* = 18.9, 1.3 Hz, 1 H, H 10), 3.03 (s, 1 H, 14-OH), 3.01 (d, *J* = 18.9 Hz, 1 H, H 10), 2.34 (br d, *J* = 14.7 Hz, 1 H, H 8), 2.18 (dd, *J* = 14.7, 4.0 Hz, 1 H, H 8), 2.02 (br s, 1 H, 4'-OH), 1.90 (dd, *J* = 13.2, 4.7 Hz, 1 H, H 2'), 1.79 (dd, *J* = 13.2, 3.3 Hz, 1 H, H 2'), 1.30 (d, *J* = 6.5 Hz, 3 H, H 6'); ¹³C NMR

δ 213.7 (C 13), 187.1 (C 5), 186.7 (C 12), 161.0 (C 4), 157.0 (C 2'), 156.1 (C 6), 155.6 (C 11), 155.2 (OCONH), 148.5 (C 4'), 135.8 (C 2), 135.5 (C12a), 133.5 (C 6a), 133.4 (C 10a), 132.5 (C 1'), 128.4 (C 6'), 120.8 (C 4a), 119.9 (C 1), 118.5 (C 3), 115.7 (C 5'), 111.6 (C 5a), 111.4 (C 11a), 105.1 (C 3'), 100.7 (C 1'), 76.6 (C 9), 69.8 (C 7), 69.6 (C 4'), 67.2 (C 5'), 65.5 (C 14), 61.1 (CH₂O), 56.7 (4-OCH₃), 56.0 (2'-OCH₃), 47.1 (C 3'), 35.6 (C 8), 34.0 (C 10), 30.2 (C 2), 16.8 (C 6'); MS (FAB⁺) m/z 753 (MH⁺, 0.3%); HRMS (FAB⁺) calc. for C₃₆H₃₇N₂O₁₆ (MH⁺) m/z 753.2143, found 753.2100; Anal (C₃₆H₃₆N₂O₁₆) C, H, N.

Example 2F. Preparation of 4-({(2-methoxy-4-

10 nitrobenzyl)oxy]carbonyl}amino)benzyl doxorubicin carbamate (42).

2-Methoxy-4-nitrobenzyl 4-({(tert-butyl(dimethyl)silyl)oxy}methyl)phenylcarbamate (39). Et₃N (0.40 mL, 2.84 mmol) was added to a stirred suspension of carbonate 37 (0.90 g,

2.58 mmol), 4-({(tert-butyl(dimethyl)silyl)oxy}methyl)aniline (9) (0.64 g, 2.71 mmol), HOBT (0.35 g, 2.58 mmol), and 4 Å molecular sieves (900 mg) in THF (80 mL) and the

15 mixture stirred at 20 °C for 16 h. The solvent was evaporated and the residue partitioned between EtOAc (100 mL) and water (100 mL). The organic fraction was washed with 1 M HCl (2 × 40 mL), water (100 mL), brine (50 mL), dried, and the solvent evaporated. The residue was purified by chromatography, eluting with 20% EtOAc/light petroleum, to give

39 (0.89 g, 77%) as a white solid, mp (EtOAc/light petroleum) 120-122 °C; ¹H NMR δ 7.84 (dd, J = 8.3, 2.1 Hz, 1 H, H 5'), 7.72 (d, J = 2.1 Hz, 1 H, H 3'), 7.51 (d, J = 8.3 Hz, 1 H, H 6'), 7.35 (d, J = 8.3 Hz, 2 H, H 2, H 6), 7.26 (d, J = 8.3 Hz, 2 H, H 3, H 5), 6.76 (br s, 1 H, OCONH), 5.30 (s, 2 H, CH₂O), 4.69 (s, 2 H, CH₂OSi), 3.93 (s, 3 H, OCH₃), 0.92 (s, 9 H, SiC(CH₃)₃), 0.09 (s, 6 H, Si(CH₃)₂); ¹³C NMR δ 157.3 (C 2'), 153.0 (OCONH), 148.7 (C 4'), 137.0 (C 4), 136.4 (C 1), 132.1 (C 1'), 128.7 (C 6'), 126.9 (C 3, C 5), 118.6 (C 2, C 6),

25 115.7 (C 5'), 105.2 (C 3'), 64.6 (CH₂O), 61.4 (CH₂O), 56.0 (OCH₃), 26.9 (SiC(CH₃)₃), 18.4 (SiC(CH₃)₃), -5.2 (Si(CH₃)₂); Anal. (C₂₂H₃₀N₂O₆Si) C, H, N.

2-Methoxy-4-nitrobenzyl 4-(hydroxymethyl)phenylcarbamate (40). 1 M HCl (4 mL, 4

30 mmol) was added to a stirred solution of silyl ether 39 (0.89 g, 0.2 mmol) in MeOH (10 mL) and stirred at 20 °C for 1 h. The solution was poured into brine (50 mL) and extracted with EtOAc (3 × 50 mL). The combined organic fraction was washed with water (50 mL), dried, and the solvent evaporated. The residue was purified by chromatography, eluting

- with a gradient (20-50%) EtOAc/light petroleum, to give **40** (628 mg, 95%) as a white solid, mp (EtOAc/light petroleum) 164-165 °C; ¹H NMR [(CD₃)₂SO] δ 9.83 (br s, 1 H, OCONH), 7.90 (dd, *J* = 8.3, 2.1 Hz, 1 H, H 5'), 7.80 (d, *J* = 2.1 Hz, 1 H, H 3'), 7.63 (d, *J* = 8.3 Hz, 1 H, H 6'), 7.41 (d, *J* = 8.4 Hz, 2 H, H 2, H 6), 7.22 (d, *J* = 8.4 Hz, 2 H, H 3, H 5), 5.21 (s, 2 H, CH₂O), 5.07 (t, *J* = 5.6 Hz, 1 H, OH), 4.41 (t, *J* = 5.6 Hz, 2 H, CH₂O), 3.97 (s, 3 H, OCH₃); ¹³C NMR [(CD₃)₂SO] δ 157.0 (C 2'), 153.0 (OCONH), 148.2 (C 4'), 137.4 (C 4), 136.7 (C 1), 132.3 (C 1'), 128.8 (C 6'), 127.0 (C 3, C 5), 117.9 (C 2, C 6), 115.5 (C 5'), 105.4 (C 3'), 62.5 (CH₂O), 60.4 (CH₂O), 56.0 (OCH₃); Anal. (C₁₆H₁₆N₂O₆) C, H, N.
- 10 **4-(((2-Methoxy-4-nitrobenzyl)oxy)carbonyl)amino)benzyl 4-nitrophenyl carbonate (41)**. A solution of 4-nitrophenylchloroformate (205 mg, 1.02 mmol) in THF (5 mL) was added dropwise to a stirred solution of alcohol **40** (282 mg, 0.85 mmol) and Et₃N (142 μL, 1.02 mmol) in THF/DMF (1:1, 30 mL) the solution stirred at 20 °C for 16 h. The solvent was evaporated and the residue was purified by chromatography, eluting with 10% EtOAc/DCM, to give **41** (238 mg, 56 %) as a white powder mp (EtOAc/DCM) 144-146 °C; ¹H NMR [(CD₃)₂SO] δ 10.01 (s, 1 H, OCONH), 8.31 (ddd, *J* = 9.1, 3.4, 2.2 Hz, 2 H, H 3, H 5), 7.91 (dd, *J* = 8.3, 2.2 Hz, 1 H, H 5'), 7.81 (d, *J* = 2.2 Hz, 1 H, H 3''), 7.64 (d, *J* = 8.3 Hz, 1 H, H 6''), 7.56 (ddd, *J* = 9.1, 3.4, 2.2 Hz, 2 H, H 2, H 6), 7.53 (br d, *J* = 8.6 Hz, 2 H, H 3', H 5'), 7.41 (br d, *J* = 8.6 Hz, 2 H, H 2', H 6'), 5.24 (s, 4 H, 2 × CH₂O), 3.98 (s, 3 H, OCH₃); ¹³C NMR [(CD₃)₂SO] δ 157.0 (C 2''), 155.2 (OCO₂), 153.0 (OCONH), 151.9 (C 1), 148.2 (C 4''), 145.1 (C 4), 139.4 (C 1), 132.2 (C 1'), 129.6 (C 2', C 6'), 128.9 (C 6''), 128.5 (C 4'), 125.3 (C 2, C 6), 122.6 (C 3, C 5), 118.0 (C 3', C 5'), 115.5 (C 5''), 105.5 (C 3''), 70.2 (CH₂O), 60.5 (CH₂O), 56.2 (OCH₃); MS (FAB⁺) *m/z* 498 (MH⁺, 0.5%); HRMS (FAB⁺) calc. for C₂₃H₂₀N₃O₁₀ (MH⁺) *m/z* 498.1149, found 498.1151. Anal. (C₂₃H₁₉N₃O₁₀) C, H, N.

- 4-(((2-Methoxy-4-nitrobenzyl)oxy)carbonyl)amino)benzyl doxorubicin carbamate (42)**. A solution of carbonate **41** (52 mg, 103 μmol) in DMF (2 mL) was added dropwise to a stirred solution of doxorubicin (**13**) (45 mg, 86 μmol) and Et₃N (15 μL, 103 μmol) in DMF (5 mL) at 20 °C and the solution stirred at 20 °C for 24 h. The solvent was evaporated and the residue purified by chromatography, eluting with a gradient (0-5%) of MeOH/DCM, to give **42** (61 mg, 80%) as a red solid, mp (DCM) 128-131 °C; ¹H NMR

$[(CD_3)_2SO]$ δ 14.01 (s, 1 H, 6-OH), 13.25 (s, 1 H, 11-OH), 9.88 (s, 1 H, OCONH), 7.87-7.90 (m, 3 H, H 1, H 2, H 5'''), 7.79 (d, $J = 2.2$ Hz, 1 H, H 3'''), 7.59-7.63 (m, 2 H, H 3, H 6'''), 7.41 (d, $J = 8.3$ Hz, 2 H, H 3'', H 5''), 7.22 (d, $J = 8.3$ Hz, 2 H, H 2'', H 6''), 6.81 (d, $J = 8.0$ Hz, 1 H, OCONH), 5.44 (s, 1 H, H 7), 5.21 (d, $J = 3.0$ Hz, 1 H, H 1'), 5.19 (s, 2 H, CH₂O), 4.91-4.94 (m, 1 H, 9-OH), 4.87 (s, 2 H, CH₂O), 4.83 (dd, $J = 6.3, 5.9$ Hz, 1 H, 14-OH), 4.69 (d, $J = 5.7$ Hz, 1 H, 4-OH), 4.58 (d, $J = 6.0$ Hz, 2 H, H 14), 4.12-4.18 (m, 1 H, H 5'), 3.97 (s, 3 H, OCH₃), 3.95 (s, 3 H, OCH₃), 3.68-3.75 (m, 1 H, H 3'), 3.43-3.47 (m, 1 H, H 4'), 2.99 (d, $J = 18.4$ Hz, 1 H, H 10), 2.92 (d, $J = 18.4$ Hz, 1 H, H 10), 2.20 (br d, $J = 14.1$ Hz, 1 H, H 8), 2.12 (dd, $J = 14.1$ Hz, 1 H, H 8), 1.85 (dt, $J = 12.8, 3.7$ Hz, 1 H, H 2'), 1.47 (dd, $J = 12.8, 4.1$ Hz, 1 H, H 2'), 1.13 (d, $J = 6.5$ Hz, 3 H, H 6'); ¹³C NMR $[(CD_3)_2SO]$ δ 213.7 (C 13), 186.4 (C 5), 186.3 (C 12), 160.7 (C 4), 157.0 (C 2'''), 156.0 (C 6), 155.2 (C 11), 154.4 (OCONH), 152.9 (OCONH), 148.2 (C 4'''), 138.4 (C 4''), 136.1 (C 2), 135.4 (C 12a), 134.6 (C 6a), 134.0 (C 10a), 132.2 (C 1''), 131.0 (C 1'''), 128.9 (C 2'''), 128.6 (C 2'', C 6''), 119.9 (C 4a), 119.6 (C 1), 118.9 (C 3), 117.9 (C 3'', C 5''), 115.4 (C 5'''), 110.7 (C 5a), 110.6 (C 11a), 105.4 (C 3'''), 100.2 (C 1'), 74.9 (C 9), 69.8 (C 7), 67.9 (C 4'), 66.6 (C 5'), 64.8 (C 14), 63.6 (CH₂O), 60.4 (CH₂O), 56.5 (OCH₃), 56.2 (OCH₃), 47.0 (C 3'), 36.5 (C 8), 32.0 (C 10), 29.7 (C 2'), 16.9 (C 6'); MS (FAB⁺) m/z 902 (MH⁺, 0.2%); Anal. (C₄₄H₄₃N₃O₁₈·H₂O) C, H, N, calc 4.6, found 5.6%.

20 Example 2G. Preparation of 2-methoxy-4-nitrobenzyl 2-[(9-amino-5-methyl-4-acridinyl)carbonyl]amino}ethyl(methyl)carbamate (46).

2-Methoxy-4-nitrobenzyl methyl{2-[(trifluoroacetyl)amino]ethyl}carbamate (43). A solution of 2-methoxy-4-nitrobenzyl alcohol (29) (183 mg, 1.0 mmol) and DIEA (0.19 mL, 1.2 mmol) in DCM (2 mL) was added dropwise to a solution of triphosgene (104 mg, 0.35 mmol) in DCM (1.5 mL) over 30 min at °C. The reaction was stirred at 5 °C for 1 h, then a solution of 2,2,2-trifluoro-*N*-[2-(methylamino)ethyl]acetamide trifluoroacetate (282 mg, 1.0 mmol) and DIEA (0.38 mL, 2.4 mmol) in DCM (2 mL) was added and the solution stirred at 20 °C for 16 h. The solvent was evaporated and the residue purified by chromatography, eluting with 40% EtOAc/petroleum ether, to give 43 (371 mg, 94%) as a white solid, mp 107-109 °C; ¹H NMR δ 7.86 (dd, $J = 8.4, 2.2$ Hz, 1 H, H 5), 7.72 (d, $J = 2.2$ Hz, 1 H, H 3), 7.56 (br s, 1 H, CONH), 7.44 (d, $J = 8.4$ Hz, 1 H, H 6), 5.23 (s, 2 H, CH₂O), 3.95 (s, 3 H, OCH₃), 3.58 (br s, 4 H, 2 CH₂N), 3.03 (s, 3 H, NCH₃); ¹³C NMR δ 157.7 (C 2), 157.4 (q, J

- 50 -

= 37 Hz, COCF_3), 157.1 (OCON), 148.6 (C 4), 132.2 (C 1), 128.1 (C 6), 115.7 (C 5), 115.7 (q, $J = 288$ Hz, CF_3), 105.2 (C 3), 62.3 (CH_2O), 56.0 (OCH_3), 47.9 (CH_2N), 39.5 (CH_2N), 35.0 (NCH_3); Anal. ($\text{C}_{14}\text{H}_{16}\text{F}_3\text{N}_3\text{O}_6$) C, H, N.

5 **2-Methoxy-4-nitrobenzyl 2-aminoethyl(methyl)carbamate (44).** A solution of carbamate **43** (948 mg, 2.5 mmol), Cs_2CO_3 (4.0 g, 12.0 mmol) and water (5 mL) in methanol (20 mL) was stirred at 20 °C for 8 h. The pH was adjusted to 10 with 1 M HCl, water (50 mL) was added, and the solution was extracted with DCM (3 50 mL). The combined organic phase was dried and the solvent was evaporated to give **44** (578 mg, 83%) as a colorless oil which
10 was used directly, ^1H NMR δ 7.85 (dd, $J = 2.0, 8.2$ Hz, 1 H, H 5), 7.72 (d, $J = 2.0$ Hz, 1 H, H 3), 7.47 (d, $J = 8.2$ Hz, 1 H, H 6), 5.23 (s, 2 H, CH_2O), 3.95 (s, 3 H, OCH_3), 3.37-3.40 (m, 2 H, CH_2), 2.99-3.02 (m, 3 H, CH_3N), 2.87-2.90 (m, 2 H, CH_2), NH_2 not observed; ^{13}C NMR δ 153.0, 148.5, 132.9, 128.3, 127.8, 115.7, 105.1, 61.7, 56.0, 52.2, 40.1, 35.2.

15 **2-Methoxy-4-nitrobenzyl 2-[(9-chloro-5-methyl-4-acridinyl)carbonyl]aminoethyl(methyl)carbamate (45).** A stirred suspension of 5-methyl-9-oxo-9,10-dihydro-4-acridinecarboxylic acid (**16**) (507 mg, 2.0 mmol) in SOCl_2 (30 mL) containing DMF (2 drops) was heated gently under reflux until homogeneous and then for a further 45 min. The solution was evaporated below 40 °C, and the residue
20 azeotroped with benzene. The residue was dissolved in DCM (30 mL), cooled to 5 °C, DIEA (1 mL, 6 mmol) and **44** added, and the solution stirred at 20 °C for 30 min. The solvent was evaporated and the residue purified by chromatography, eluting with 75% EtOAc/light petroleum, to give **45** (255 mg, 50 %) as a yellow solid, mp (EtOAc/light petroleum) 60-65°C; ^1H NMR [$(\text{CD}_3)_2\text{SO}$] δ 11.92-11.96 (m, 1 H, NH), 8.91-9.00 (m, 1 H),
25 8.53-8.61 (m, 1 H), 8.26-8.29 (m, 1 H), 7.66-7.75 (m, 2 H), 7.51-7.60 (m, 2 H), 7.17-7.30 (m, 2 H), 5.15 (s, 2 H, CH_2O), 3.89 (s, 3 H, OCH_3), 3.72-3.75 (m, 2 H, CH_2), 3.62-3.65 (m, 2 H, CH_2), 3.09 (s, 3 H, CH_3N), 2.75 (s, 3 H, CH_3); ^{13}C NMR [$(\text{CD}_3)_2\text{SO}$] δ 165.9, 156.3, 146.6, 145.0, 143.3, 135.9, 135.6, 132.4, 131.6, 128.8, 128.7, 128.4, 128.2, 127.7, 127.5, 126.5, 124.2, 123.8, 123.0, 115.5, 104.3, 61.5, 55.8, 49.0, 38.0, 35.2, 18.9.

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2-Methoxy-4-nitrobenzyl 2-[(9-amino-5-methyl-4-acridinyl)carbonyl]aminoethyl(methyl)carbamate (46). A solution of chloride **45** (100

mg, 0.17 mmol) in dry phenol (1.2 g, 13 mmol) was heated at 50 °C. Dry ammonia was bubbled through the solution while the temperature was raised from 50 to 120°C. Addition of ammonia was continued for 15 min, then the mixture was cooled and diluted with excess 40% aqueous NaOH. Prolonged cooling gave a solid that was purified by chromatography, 5 eluting with 20% MeOH/DCM, to give 46 (80 mg, 92 %) as a yellow solid, mp (MeOH/DCM) 245-248 °C; ¹H NMR [(CD₃)₂SO] δ 12.72-12.74 (m, 1 H, NH), 8.55-8.66 (m, 2 H), 8.28-8.24 (m, 1 H), 8.10-8.14 (m, 2 H), 7.66-7.09 (m, 4 H), 5.00 (s, 2 H, CH₂O), 3.73 (s, 3 H, OCH₃), 3.65 (m, 4 H, 2 CH₂), 2.95 (s, 3 H, CH₃N), 2.51 (s, 3 H, CH₃); ¹³C NMR [(CD₃)₂SO] δ 165.9, 155.7, 155.2, 152.2, 147.1, 146.2, 145.4, 134.3, 134.0, 132.2, 10 130.9, 127.2, 126.8, 126.6, 121.7, 121.1, 120.4, 114.7, 113.0, 111.7, 104.4, 60.8, 55.7, 48.4, 36.7, 34.3, 18.5. Anal. (C₂₇H₁₇N₃O₆) C, H, N.

Example 2H. Preparation of 2-methoxy-4-nitrobenzyl bis(3-[(5-methyl-4-acridinyl)carbonyl]amino)propyl)carbamate (51).

15 **2,2,2-Trifluoro-N-[3-({3-[(trifluoroacetyl)amino]propyl}amino)propyl]acetamide trifluoroacetate (47).** Water (1.2 mL, 70 mmol) was added to a stirred solution of *N*-(3-aminopropyl)-1,3-propanediamine (4.0 g, 30.5 mmol) and ethyl trifluoroacetate (15.0 g, 105 mmol) in MeCN (60 mL) and the solution heated at reflux for 3 h. The solution was cooled, the solvent evaporated, and the residue was triturated with DCM (100 mL). The 20 suspension was filtered to give 47 (1.20 g, 90%) as white solid, mp (DCM) 175-178 °C; ¹H NMR [(CD₃)₂SO] δ 9.55 (s, 2 H, NH₂⁺), 8.45 (br s, 2 H, 2 CONH), 3.24-3.28 (m, 4 H, 2 CH₂N), 2.90-2.94 (m, 4 H, 2 CH₂N), 1.76-1.84 (m, 4 H, 2 CH₂); Anal. (C₁₂H₁₆F₉N₃O₄) C, H, N.

25 **2-Methoxy-4-nitrobenzyl 3-[(trifluoroacetyl)amino]propyl(6,6,6-trifluoro-5-oxohexyl)carbamate (48).** A solution of 2-methoxy-4-nitrobenzyl alcohol (29) (183 mg, 1.0 mmol) and DIEA (0.19 mL, 1.2 mmol) in DCM (2 mL) was added dropwise to a solution of triphosgene (104 mg, 0.35 mmol) in DCM (1.5 mL) over 30 minutes at 5 °C and the solution stirred for 1 h. The solution was added dropwise to a suspension of 30 bistrifluoroacetamide 47 (437 mg, 1.0 mmol) and DIEA (0.38 mL, 2.4 mmol) in DCM (2 mL) and the solution stirred at 20 °C for 16 h. The solvent was evaporated and the residue purified by chromatography, eluting with 40% EtOAc/light petroleum, to give 48 (373 mg,

70%) as a white solid, mp (EtOAc/light petroleum) 133-135°C; ¹H NMR [(CD₃)₂SO] δ 9.41 (s, 2 H, 2 CONH), 7.85 (dd, *J* = 8.0, 2.0 Hz, 1 H, H 5), 7.78 (d, *J* = 2.0 Hz, 1 H, H 3), 7.50 (d, *J* = 8.0 Hz, 1 H, H 6), 5.12 (s, 2 H, CH₂O), 3.95 (s, 3 H, OCH₃), 3.15-3.35 (m, 8 H, 4 CH₂N), 1.74 (m, 4 H, 2 CH₂). Anal. (C₁₉H₂₂F₆N₄O₇) C, H, N.

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2-Methoxy-4-nitrobenzyl bis(3-((5-methyl-4-

acridinyl)carbonyl]amino}propyl)carbamate (51). A solution of carbamate 48 (107 mg, 0.2 mmol), Cs₂CO₃ (1.0 g, 3.0 mmol) and water (1 mL) in methanol (4 mL) was stirred at 20 °C for 8 hrs. The pH was adjusted to 10 with 1M HCl, water (50 mL) added, and the

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solution was extracted with DCM (3 50 mL). The combined organic fraction was dried, the solvent was evaporated to give crude 2-methoxy-4-nitrobenzyl bis(3-

aminopropyl)carbamate 49). 4-(1*H*-Imidazol-1-ylcarbonyl)-5-methylacridine (50) [S. A.

Gamage, J. A. Spicer, G. J. Atwell, G. J. Finlay, B. C. Baguley, W. A. Denny, *J. Med.*

Chem., 1999, 42, 2383-2393] (104 mg, 0.36 mmol) was added to a solution of the crude

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carbamate 49 in THF (10 mL) at 5 °C and the reaction mixture was stirred at 20 °C for 8 hrs. The solvent was evaporated, and the residue was purified by chromatography on

alumina-90, eluting with 2%MeOH/45%EtOAc/DCM, to give 51 (85 mg, 64%) as a yellow

solid, mp (EtOAc/DCM) 88-90 °C; ¹H NMR δ 11.87 (s, 1 H, NH), 11.81 (s, 1 H, NH), 8.90 (s, 2 H), 8.70 (s, 1 H), 8.67 (s, 1 H), 8.03 (m, 2 H), 7.78 (m, 2 H), 7.58 (m, 4 H), 7.40 (m, 2

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H), 7.28 (d, *J* = 2.0 Hz, 1 H, H 3"), 7.05 (d, *J* = 8.4 Hz, 1 H, H 6"), 6.97 (dd, *J* = 8.4, 2.0 Hz, 1 H, H 5"), 5.01 (s, 2 H, CH₂O), 3.71 (m, 7 H), 3.58 (m, 4 H), 2.81 (s, 3 H, CH₃), 2.70

(s, 3 H, CH₃), 2.11 (m, 4 H); ¹³C NMR δ 166.1, 156.3, 155.6, 147.8, 146.9 (2), 146.7, 145.2, 145.1, 137.8, 135.7 (2), 135.3, 135.1 (2), 132.3 (2), 132.2 (2), 130.9, 128.2, 127.9, 126.9

(2), 126.5, 126.3, 126.2, 126.1 (2), 125.7 (2), 125.3 (2), 115.1, 104.2, 61.6, 55.6, 45.8, 45.1,

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37.7, 37.1, 29.2, 28.5, 18.9, 18.7; Anal. (C₄₅H₄₂N₆O₇·½H₂O) C, H, N.

Example 2I. Preparation of 2-methoxy-4-nitrobenzyl 2-[(5,8-dihydroxy-4-[[2-

(methyl{[(2-methoxy-4-nitrobenzyl)oxy]carbonyl}amino)ethyl]amino}-9,10-dioxo-

9,10-dihydro-1-anthracenyl)amino]ethyl(methyl)carbamate (53). A solution of 1,4-

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difluoro-5,8-dihydroxyanthracene-9,10-dione (22) (1.0 g, 3.6 mmol) and 2-methoxy-4-

nitrobenzyl 2-aminoethyl(methyl)carbamate (44) (0.8 g, 2.7 mmol) in pyridine (20 mL)

was stirred at 20 °C for 48 h. The solvent was evaporated and residue was purified by

column chromatography, eluting with a gradient (50-85%) of EtOAc/DCM, to give:

(i) starting material (22) (0.15 g, 15%) and:

(ii) 2-methoxy-4-nitrobenzyl 2-[(4-fluoro-5,8-dihydroxy-9,10-dioxo-9,10-dihydro-1-anthracenyl)amino]ethyl(methyl)carbamate (52) (0.54 g, 37%) as a purple solid mp

- 5 (EtOAc/DCM) 122-125 °C; $^1\text{H NMR}$ δ 13.05-13.77 (m, 2 H, 2 \times OH), 9.84-10.07 (br d, 1 H, NH), 6.99-7.83 (m, 7 H), 5.27 (s, 2 H, CH_2O), 3.95 (s, 3 H, OCH_3), 3.63 (m, 4 H), 3.09 (s, 3 H, NCH_3); HRMS (FAB $^+$) calc. for $\text{C}_{26}\text{H}_{22}\text{FN}_3\text{O}_9$ (M^+) m/z 539.1340, found 539.1331; and:

- 10 (iii) 53 (120 mg, 5%) as a blue solid, $^1\text{H NMR}$ δ 13.15-13.36 (m, 2 H, 2 \times OH), 10.29-10.42 (m, 2 H, 2 \times NH), 6.97-7.82 (m, 10 H), 5.26 (s, 2 H, CH_2O), 5.12 (s, 2 H, CH_2O), 3.95 (s, 3 H, OCH_3), 2.78 (s, 3 H, OCH_3), 3.50 (br, 8 H), 3.05 (s, 6 H, 2 \times NCH_3); HRMS (FAB $^+$) calc. for $\text{C}_{38}\text{H}_{38}\text{N}_6\text{O}_{14}$ (M^+) m/z 802.2446, found 802.2446.

Example 2J. Preparation of 2-methoxy-4-nitrobenzyl 2-[(5,8-dihydroxy-4-((2-(2-hydroxyethyl)amino)ethyl)amino)-9,10-dioxo-9,10-dihydro-1-anthracenyl]amino}ethyl(methyl)carbamate (55).

A solution of fluoride 52 (0.54 g, 1.0 mmol) and 2-(2-aminoethylamino)ethanol (2.0 g, 19 mmol) in pyridine (20 mL) was stirred at 20 °C for 54 h. The solvent was evaporated and residue was purified by column chromatography, eluting with a gradient (50-85%) of

- 20 EtOAc/light petroleum followed by (2-10%) MeOH/EtOAc, to give:

(i) 2-methoxy-4-nitrobenzyl 2-[[8,11-dihydroxy-4-(2-hydroxyethyl)-7,12-dioxo-1,2,3,4,7,12-hexahydronaphtho[2,3-f]quinoxalin-6-yl]amino}ethyl(methyl)carbamate (54) (0.1 g, 16%) as a blue solid; mp (DCM/light petroleum) 221-224 °C; $^1\text{H NMR}$ δ 13.43-

- 25 14.25 (m, 2 H, 2 \times OH), 10.89-11.29 (m, 2 H, 2 \times NH), 6.96-7.77 (m, 6 H), 6.20 (s, 1 H), 4.80 (s, 2 H, CH_2O), 3.94 (s, 3 H, OCH_3), 3.65 (m, 12 H), 2.97 (s, 3 H, NCH_3); and:

(ii) 55 (0.45 g, 72%) as a blue oil, $^1\text{H NMR}$ δ 13.26-13.51 (m, 2 H, 2 \times OH), 10.44-10.50 (m, 2 H, 2 \times NH), 7.02-7.88 (m, 7 H), 5.28 (s, 2 H, CH_2O), 3.96 (s, 3 H, OCH_3), 3.39-3.65 (m, 12 H), 3.07 (s, 3 H, NCH_3), NH, OH not observed; HRMS (FAB $^+$) calc. for $\text{C}_{30}\text{H}_{34}\text{N}_5\text{O}_{10}$ (MH^+) m/z 624.2306, found 624.2297..

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Example 3A. Preparation of 1-(4-nitrophenyl)ethyl 4-[bis(2-chloroethyl)amino]phenylcarbamate (59).

1-(4-Nitrophenyl)ethyl 4-[bis(2-hydroxyethyl)amino]phenylcarbamate (58). Pyridine (320 mL, 3.95 mmol) was added dropwise to a stirred solution of 1-(4-nitrophenyl)ethanol 56 (660 mg, 3.95 mmol) and triphosgene (410 mg, 1.38 mmol) in THF (50 mL) at 5 °C and the suspension stirred at 5 °C for 1 h. A solution of *N*¹,*N*¹-bis(2-hydroxyethyl)-1,4-benzenediamine (57) (prepared by catalytic hydrogenation of *N,N*-bis-(2-hydroxyethyl)-4-nitroaniline (30) (0.85 g, 4.34 mmol) with Pd/C under H₂ (60 psi) in EtOH) in THF (10 mL) and DMF (20 mL) was added and the mixture stirred at 20 °C for 16 h. The solvent was evaporated and the residue purified by chromatography, eluting with EtOAc to give 58 (1.01 g, 66 %) as a brown oil, ¹H NMR δ 8.19 (d, *J* = 8.8 Hz, 2 H, H 3', H 5'), 7.52 (br d, *J* = 8.8 Hz, 2 H, H 2', H 6'), 7.16 (d, *J* = 9.0 Hz, 2 H, H 2, H 6), 6.69 (br s, 1 H, OCONH), 6.62 (d, *J* = 9.0 Hz, 2 H, H 3, H 5), 5.90 (q, *J* = 6.6 Hz, 1 H, CHO), 3.76-3.80 (m, 4 H, 2 × CH₂O), 3.55 (br s, 2 H, 2 × OH), 3.48-3.51 (m, 4 H, 2 × CH₂N), 1.58 (d, *J* = 6.6 Hz, 3 H, CH₃); ¹³C NMR δ 153.1, 149.4, 147.4, 144.9, 127.3, 126.6 (2), 123.8 (2), 121.4 (2), 113.3 (2), 71.9, 60.6 (2), 55.3 (2), 22.5; MS (FAB⁺) *m/z* 390 (MH⁺, 25%); HRMS (FAB⁺) calc. for C₁₉H₂₄N₃O₆ (MH⁺) *m/z* 390.1665, found 390.1656.

1-(4-nitrophenyl)ethyl 4-[bis(2-chloroethyl)amino]phenylcarbamate (59). Methanesulphonyl chloride (600 μL, 7.7 mmol) was added dropwise to a stirred solution of 58 (1.0 g, 2.57 mmol) in pyridine (20 mL) at 20 °C and the solution stirred for 1 h. The solvent was evaporated and the residue partitioned between DCM/water (100 mL). The aqueous fraction was extracted with DCM (2 × 50 mL) and the combined organic fraction washed with brine (50 mL), dried and the solvent evaporated. The residue was dissolved in DMF (15 mL), LiCl (0.65 g, 15.4 mmol) added, and the mixture stirred at 80 °C for 3 h. The solvent was evaporated and the residue partitioned between EtOAc/water (200 mL). The aqueous fraction was extracted with EtOAc (2 × 50 mL). The combined organic fraction was washed with brine (50 mL), dried, and the solvent evaporated. The residue was purified by chromatography, eluting with 25% EtOAc/light petroleum, to give 59 (1.0 g, 92 %) as a tan oil, ¹H NMR δ 8.20 (ddd, *J* = 8.9, 2.2, 1.9 Hz, 2 H, H 3', H 5'), 7.53 (br d, *J* = 8.9 Hz, 2 H, H 2', H 6'), 7.23 (br d, *J* = 9.0 Hz, 2 H, H 2, H 6), 6.64 (ddd, *J* = 9.0, 3.4, 2.1 Hz, 2 H, H 3, H 5), 6.61 (br s, 1 H, OCONH), 5.92 (q, *J* = 6.6 Hz, 1 H, CHO), 3.67-3.71 (m, 4 H, 2 × CH₂N), 3.58-3.62 (m, 4 H, 2 × CH₂Cl), 1.59 (d, *J* = 6.6 Hz, 3 H, CH₃); ¹³C NMR δ 152.9, 149.3, 147.4, 142.9, 128.0, 126.6 (2), 123.8 (2), 121.4 (2), 112.7 (2), 71.9,

53.6 (2), 40.4 (2), 22.4; MS (FAB⁺) m/z 429 (M⁺, 5%), 427 (10), 425 (15); HRMS (FAB⁺) calc. for C₁₉H₂₁³⁵Cl₂N₃O₄ (M⁺) m/z 425.0909, found 425.0901; calc. for C₁₉H₂₁³⁵Cl³⁷CIN₃O₄ (M⁺) m/z 427.0880, found 427.0882; calc. for C₁₉H₂₁³⁷Cl₂N₃O₄ (M⁺) m/z 429.0850, found 429.0868.

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Example 3B. Preparation of 1-(4-nitrophenyl)ethyl 1-(chloromethyl)-3-[(5,6,7-

trimethoxy-1H-indol-2-yl)carbonyl]-2,3-dihydro-1H-benzo[e]indol-5-ylcarbamate

(60). A solution of 1-(4-nitrophenyl)ethanol (56) (18 mg, 0.11 mmol) in DCM (2 mL)

was added dropwise to a stirred solution of triphosgene (16 mg, 0.054 mmol) and pyridine

10 (9 μ L, 0.11 mmol) in DCM (2 mL) at 20 °C. The mixture was stirred at 20 °C for 2 h, the

solvent evaporated and the residue dissolved in THF (5 mL). A solution of 1 [G. J. Atwell,

W. R. Wilson, W. A. Denny, *Bioorg. Med. Chem. Lett.*, 1997, 7, 1483] (50 mg, 0.11 mmol)

in THF (5 mL) was added and the solution stirred at 20 °C for 16 h. The mixture was

partitioned between EtOAc (50 mL) and sat. aq. KHCO₃ solution, the organic fraction dried

15 and the solvent evaporated. The residue was purified by chromatography, eluting with 25%

EtOAc/light petroleum to give: (i) 60 (23 mg, 32%) as a tan solid mp (EtOAc/light

petroleum) 175-178 °C; ¹H NMR δ 9.49 (s, 1 H, indole-NH), 8.88 (s, 1 H, OCONH), 8.18

(d, J = 7.6 Hz, 2 H, H 3", H 5"), 7.88 (d, J = 8.3 Hz, 1 H, H 6), 7.78 (d, J = 8.3 Hz, 1 H, H

9), 7.52-7.58 (m, 3 H, H 8, H 2", H 6"), 7.45 (dd, J = 7.8, 7.5 Hz, 1 H, H 7), 7.16 (br s, 1 H,

20 H 4), 7.00 (d, J = 1.90 Hz, 1 H, H 3'), 6.87 (s, 1 H, H 4'), 6.00 (q, J = 6.6 Hz, 1 H, CHO),

4.80 (dd, J = 10.7, 1.2 Hz, 1 H, H 2), 4.65 (dd, J = 10.7, 8.8 Hz, 1 H, H 2), 4.11-4.17 (m, 1

H, CH₂Cl), 4.08 (s, 3 H, OCH₃), 3.93-3.97 (m, 4 H, OCH₃, CH₂Cl), 3.91 (s, 3 H, OCH₃),

3.45 (dt, J = 10.7, 3.3 Hz, 1 H, H 1), 1.65 (br d, J = 6.6 Hz, 3 H, CH₃); ¹³C NMR δ 160.3,

153.4, 150.2, 149.0, 147.5, 141.6, 140.6, 138.9, 133.8, 130.4, 129.7, 129.6, 127.4, 126.8

25 (2), 125.6, 125.0, 123.9, 123.8 (2), 123.6, 123.1, 122.3, 121.7, 106.5, 97.6, 72.6, 61.5, 61.1,

56.3, 54.9, 45.8, 43.4, 22.6; MS (FAB⁺) m/z 659 (MH⁺, 6%), 658 (6), 510 (1), 234 (10);

HRMS calc. for C₃₄H₃₂³⁵CIN₄O₈ (MH⁺) m/z 659.1909, found 659.1881; calc for

C₃₄H₃₂₃₇CIN₄O₈ (MH⁺) m/z 661.1879, found 661.1882; Anal. (C₃₄H₃₁CIN₄O₈) C, H, N: and

(ii) starting material. (1) (30 mg, 60 %).

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Example 3C. Preparation of 1-(2-methoxy-4-nitrophenyl)ethyl 4-[bis(2-chloroethyl)amino]phenylcarbamate (64).

2-Methoxy-4-nitrobenzaldehyde (61). PCC (0.76 g, 3.52 mmol) and 4Å molecular sieves (1.0 g) were added to a stirred solution of 2-methoxy-4-nitrobenzyl alcohol **29** (0.43 g, 2.35 mmol) in DCM (100 mL) and the suspension stirred at 20 °C for 1 h. The suspension was diluted with diethyl ether (150 mL) and the suspension filtered through Celite®, washed with diethyl ether (2 × 20 mL). The combined organic fraction was evaporated and the residue purified by chromatography, eluting with 50% EtOAc/light petroleum, to give **61** (0.42 g, 98%) as white crystals, mp (EtOAc/light petroleum) 117-119 °C; ¹H NMR δ 10.52 (s, 1 H, CHO), 7.98 (br d, *J* = 8.2 Hz, 1 H, H 5), 7.85-7.89 (m, 2 H, H 3, H 6), 4.07 (s, 3 H, OCH₃); ¹³C NMR δ 188.2 (CHO), 161.8 (C 2), 152.2 (C 4), 129.5 (C 6), 128.6 (C 1), 115.6 (C 5), 107.2 (C 5), 56.4 (OCH₃); Anal. (C₈H₇NO₄) C, H, N.

1-(2-Methoxy-4-nitrophenyl)ethanol (62). A solution of MeMgBr (3 M in diethyl ether: 3.64 mL, 10.9 mmol) was added dropwise to a stirred solution of **61** (1.80 g, 9.93 mmol) in THF (100 mL) at -78 °C and the solution stirred at -78 °C for 20 min. The solution was quenched with sat. aq NH₄Cl solution (5 mL) and allowed to warm to 20 °C. The solvent was evaporated and the residue purified by chromatography, eluting with 20% EtOAc/light petroleum, to give (i) starting material (0.38 g, 21%) and (ii) **62** (0.88 g, 45%) as a white solid, mp (EtOAc/light petroleum) 63-65 °C; ¹H NMR δ 7.86 (dd, *J* = 8.4, 2.1 Hz, 1 H, H 5), 7.70 (d, *J* = 2.1 Hz, 1 H, H 3), 7.58 (d, *J* = 8.4 Hz, 1 H, H 6), 5.19 (dq, *J* = 6.4, 4.7 Hz, 1 H, CHOCO), 3.96 (s, 3 H, OCH₃), 2.34 (d, *J* = 4.7 Hz, 1 H, OH), 1.48 (d, *J* = 6.4 Hz, 3 H, CH₃); ¹³C NMR δ 156.4 (C 2), 148.0 (C 4), 141.3 (C 1), 126.3 (C 6), 116.2 (C 5), 105.3 (C 3), 65.5 (CHOCO), 55.9 (OCH₃), 23.1 (CH₃); Anal. (C₉H₁₁NO₄) C, H, N.

1-(2-Methoxy-4-nitrophenyl)ethyl 4-[bis(2-hydroxyethyl)amino]phenylcarbamate (63). Pyridine (178 µL, 2.20 mmol) was added dropwise to a stirred solution of ethanol **62** (430 mg, 2.20 mmol) and triphosgene (229 mg, 0.77 mmol) in THF (50 mL) at 5 °C and the suspension stirred at 5 °C for 1 h. A solution of *N*¹,*N*¹-bis(2-hydroxyethyl)-1,4-benzenediamine **57** (476 mg, 2.42 mmol) with Pd/C under H₂ (60 psi) in EtOH in THF (10 mL) and DMF (10 mL) was added and the mixture stirred at 20 °C for 10 days. The solvent was evaporated and the residue purified by chromatography, eluting with EtOAc to give **63** (860 mg, 93%) as a tan foam, ¹H NMR δ 9.42 (br s, 1 H, OCONH), 7.91 (dd, *J* = 8.4, 2.1 Hz, 1 H, H 5), 7.79 (d, *J* = 2.1 Hz, 1 H, H 3), 7.60 (d, *J* = 8.4 Hz, 1 H, H 6), 7.18 (d, *J* = 9.0

Hz, 2 H, H 2', H 6'), 6.59 (d, $J = 9.0$ Hz, 2 H, H 3', H 5'), 6.00 (q, $J = 6.5$ Hz, 1 H, CHOCO), 4.69 (br s, 2 H, $2 \times$ OH), 3.98 (s, 3 H, OCH₃), 3.48-3.53 (m, 4 H, $2 \times$ CH₂O), 3.32-3.37 (m, 4 H, $2 \times$ CH₂N), 1.46 (d, $J = 6.5$ Hz, 3 H, CH₃); ¹³C NMR δ 155.7 (C 2), 152.6 (OCONH), 147.4 (C 4), 144.0 (C 4'), 138.7 (C 1'), 127.3 (C 1), 125.7 (C 6), 120.1 (C 2', C 6'), 115.8 (C 5), 111.4 (C 3', C 5'), 105.7 (C 3), 66.0 (CHOCO), 59.7 ($2 \times$ CH₂O), 58.2 (OCH₃), 53.4 ($2 \times$ CH₂N), 20.8 (CH₃); MS (FAB⁺) m/z 420 (MH⁺, 30%); HRMS (FAB⁺) calc. for C₂₀H₂₆N₃O₇ (MH⁺) m/z 420.1771, found 420.1761.

- 1-(2-Methoxy-4-nitrophenyl)ethyl 4-[bis(2-chloroethyl)amino]phenylcarbamate (64).
- 10 Methanesulphonyl chloride (404 μ L, 5.2 mmol) was added dropwise to a stirred solution of 63 (0.73 g, 1.74 mmol) in pyridine (20 mL) at 20 °C and the solution stirred for 1 h. The solvent was evaporated and the residue partitioned between DCM/water (100 mL). The aqueous fraction was extracted with DCM ($2 \times$ 50 mL) and the combined organic fraction washed with brine (50 mL), dried and the solvent evaporated. The residue was dissolved in
- 15 DMF (20 mL), LiCl (0.44 g, 10.4 mmol) added, and the mixture stirred at 80 °C for 3 h. The solvent was evaporated and the residue partitioned between EtOAc/water (200 mL). The aqueous fraction was extracted with EtOAc ($2 \times$ 50 mL). The combined organic fraction was washed with brine (50 mL), dried, and the solvent evaporated. The residue was purified by chromatography, eluting with 20% EtOAc/light petroleum, to give 64 (0.53 g,
- 20 67 %) as a tan foam, ¹H NMR δ 7.84 (dd, $J = 8.4, 1.9$ Hz, 1 H, H 5), 7.71 (d, $J = 1.9$ Hz, 1 H, H 3), 7.50 (d, $J = 8.4$ Hz, 1 H, H 6), 7.24 (br d, $J = 9.0$ Hz, 2 H, H 2', H 6'), 6.64 (d, $J = 9.0$ Hz, 2 H, H 3', H 5'), 6.62 (br s, 1 H, OCONH), 6.19 (q, $J = 6.5$ Hz, 1 H, CHOCO), 3.95 (s, 3 H, OCH₃), 3.69 (dd, $J = 7.0, 6.4$ Hz, 4 H, $2 \times$ CH₂N), 3.60 (dd, $J = 7.0, 6.4$ Hz, 4 H, $2 \times$ CH₂Cl), 1.52 (d, $J = 6.5$ Hz, 3 H, CH₃); ¹³C NMR δ 156.0 (C 2), 152.8 (OCONH), 148.1 (C 4), 142.9 (C 4'), 138.5 (C 1'), 128.3 (C 1), 125.8 (C 6), 121.3 (C 2', C 6'), 116.0 (C 5),
- 25 112.8 (C 3', C 5'), 105.5 (C 3), 67.6 (CHOCO), 56.0 (OCH₃), 53.7 ($2 \times$ CH₂N), 40.5 ($2 \times$ CH₂Cl), 21.2 (CH₃); MS m/z 459 (M⁺, 2%), 457 (M⁺, 12), 455 (M⁺, 16), 276 (20), 231 (100); HRMS calc. for C₂₀H₂₃³⁵Cl₂N₃O₅ (M⁺) m/z 455.1015, found 455.1017; calc. for C₂₀H₂₃³⁵Cl³⁷ClN₃O₅ (M⁺) m/z 457.0985, found 457.0990; calc. for C₂₀H₂₃³⁷Cl₂N₃O₅ (M⁺) m/z
- 30 459.0956, found 459.0972.

Example 4A. Preparation of 2-(2-hydroxyethoxy)-4-nitrobenzyl 1-(chloromethyl)-3-

[(5,6,7-trimethoxy-1*H*-indol-2-yl)carbonyl]-2,3-dihydro-1*H*-benzo[*e*]indol-5-ylcarbamate (70).

- 2-Bromoethyl *tert*-butyl(dimethyl)silyl ether (65). TBDMS triflate (5.0 mL, 21.8 mmol) was added dropwise to a stirred solution of bromoethanol (1.40 mL, 19.8 mmol) and pyridine (2.4 mL, 29.7 mmol) in DCM (50 mL) at 5 °C and the solution stirred at 20 °C for 16 h. MeOH (5 mL) was carefully added, the solution stirred for 5 min and poured into sat. aq. KHCO₃ (150 mL). The mixture was extracted with DCM (3 × 80 mL), the combined organic fraction dried, and the solvent evaporated. Chromatography of the residue, eluting with 10% EtOAc/light petroleum, gave 65 (3.95 g, 83%) as a colourless oil, ¹H NMR δ 3.88 (t, *J* = 6.6 Hz, 2 H, CH₂O), 3.40 (t, *J* = 6.6 Hz, 2 H, CH₂Br), 0.90 (s, 9 H, SiC(CH₃)₃), 0.08 (s, 6 H, Si(CH₃)₂); ¹³C NMR δ 63.5 (CH₂O), 33.3 (CH₂Br), 25.8 (SiC(CH₃)₃), 18.3 (Si(CH₃)₂), -5.3 (Si(CH₃)₂); MS (CI, NH₃) *m/z* 241 (MH⁺, 1%), 239 (MH⁺, 1%), 225 (2), 223 (2), 183 (55), 181 (55), 139 (100); HRMS (CI, NH₃) calc for C₈H₂₀⁷⁹BrOSi (MH⁺) *m/z* 239.0467; found 239.0460; calc for C₈H₂₀⁸¹BrOSi (MH⁺) *m/z* 241.0446; found 241.0450.

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- Methyl 2-(2-({*tert*-butyl(dimethyl)silyl}oxy)ethoxy)-4-nitrobenzoate (67). A mixture of methyl 2-hydroxy-4-nitrobenzoate (66) (0.55 g, 2.79 mmol) and K₂CO₃ (0.58 g, 4.19 mmol) in DMF (15 mL) was stirred at 20 °C for 30 min. A solution of 65 (1.00 g, 4.19 mmol) in DMF (5 mL) was added and the mixture was stirred at 100 °C for 4 h. The solvent was evaporated and the residue partitioned between EtOAc (100 mL) and water (100 mL). The organic fraction was washed with water (2 × 50 mL), brine (50 mL), dried, and the solvent evaporated. The residue was purified by chromatography, eluting with 20% EtOAc/light petroleum, to give 67 (0.76 g, 77%) as a white solid, mp (EtOAc/light petroleum) 47-48 °C; ¹H NMR δ 7.89 (d, *J* = 1.3 Hz, 1 H, H 3), 7.81-7.85 (m, 2 H, H 5, H 6), 4.24 (t, *J* = 5.0 Hz, 2 H, CH₂O), 4.03 (t, *J* = 5.0 Hz, 2 H, CH₂O), 3.92 (s, 3 H, OCH₃), 0.88 (s, 9 H, SiC(CH₃)₃), 0.08 (s, 6 H, Si(CH₃)₂); ¹³C NMR (CDCl₃) δ 165.5 (CO₂), 158.6 (C 2), 150.4 (C 4), 131.8 (C 6), 126.7 (C 1), 115.0 (C 5), 108.3 (C 3), 71.1 (CH₂O), 61.7 (CH₂O), 52.5 (OCH₃), 25.7 (SiC(CH₃)₃), 18.3 (Si(CH₃)₂), -5.5 (Si(CH₃)₂); Anal. (C₁₆H₂₅NO₆Si) C, H, N.

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[2-(2-({*tert*-Butyl(dimethyl)silyl}oxy)ethoxy)-4-nitrophenyl]methanol (68). DIBALH (1M in DCM, 6.7 mL, 6.7 mmol) was added dropwise to a stirred solution of ester 67 (0.72

g, 2.02 mmol) in THF (50 mL) at 5 °C and the solution stirred at 5 °C for 1 h. The solution was poured into a solution of potassium sodium tartrate (1 M, 50 mL) and stirred vigorously for 30 min. The mixture was extracted with EtOAc (3 × 50 mL), the combined organic fraction washed with water (50 mL), brine (50 mL), dried and the solvent was evaporated. The residue was purified by chromatography, eluting with 20% EtOAc/light petroleum, to give **68** (0.64 g, 97%) as a white solid, mp (EtOAc/light petroleum) 89-90 °C; ¹H NMR δ 7.85 (dd, *J* = 8.2, 2.1 Hz, 1 H, H 5), 7.74 (d, *J* = 2.1 Hz, 1 H, H 3), 7.46 (d, *J* = 8.2 Hz, 1 H, H 6), 4.75 (s, 2 H, CH₂O), 4.21 (dd, *J* = 4.9, 4.4 Hz, 2 H, CH₂O), 4.01 dd, *J* = 4.9, 4.4 Hz, 2 H, CH₂O), 2.84 (br s, 1 H, OH), 0.90 (s, 9 H, SiC(CH₃)₃), 0.06 (s, 6 H, Si(CH₃)₂); ¹³C NMR δ 157.0 (C 2), 148.3 (C 4), 137.1 (C 1), 128.4 (C 6), 116.3 (C 5), 106.8 (C 3), 70.5 (CH₂O), 61.6 (CH₂O), 61.3 (CH₂O), 25.8 (SiC(CH₃)₃), 18.3 (Si(CH₃)₂), -5.4 (Si(CH₃)₂); Anal. (C₁₅H₂₅NO₅Si) C, H, N.

2-(2-([*tert*-Butyl(dimethyl)silyl]oxy)ethoxy)-4-nitrobenzyl 1-(chloromethyl)-3-[(5,6,7-trimethoxy-1*H*-indol-2-yl)carbonyl]-2,3-dihydro-1*H*-benzo[*e*]indol-5-ylcarbamate (69). A solution of triphosgene (24 mg, 80 μmol) in DCM (2 mL) was added dropwise to a stirred solution of amine **1** [G. J. Atwell, W. R. Wilson, W. A. Denny, *Bioorg. Med. Chem. Lett.*, 1997, 7, 1483] (107 mg, 230 μmol) and Et₃N (64 μL, 460 μmol) in DCM (20 mL) and stirred at 20 °C for 2 h. A solution of [2-(2-([*tert*-butyl(dimethyl)silyl]oxy)ethoxy)-4-nitrophenyl]methanol (**68**) (83 mg, 253 μmol) in DCM (5 mL) was added, followed by nBu₂Sn(OAc)₂ (2 drops) and the solution stirred at 20 °C for 24 h. The solvent was evaporated and the residue purified by chromatography, eluting with a gradient (0-20%) EtOAc/DCM, to give **69** (177 mg, 94%) as a white solid, mp (EtOAc/light petroleum) 182-185 °C; ¹H NMR δ 9.45 (s, 1 H, indole-NH), 8.93 (s, 1 H, OCONH), 7.92 (d, *J* = 8.5 Hz, 1 H, H 6), 7.76-7.83 (m, 3 H, H 9, H 3", H 5"), 7.53-7.60 (m, 2 H, H 8, H 6"), 7.47 (ddd, *J* = 8.5, 7.1, 0.8 Hz, 1 H, H 7), 7.13 (br s, 1 H, H 4), 7.01 (d, *J* = 2.2 Hz, 1 H, H 3'), 6.88 (s, 1 H, H 4'), 5.39 (s, 2 H, CH₂O), 4.81 (dd, *J* = 10.7, 1.8 Hz, 1 H, H 2), 4.67 (dd, *J* = 10.7, 8.7 Hz, 1 H, H 2), 4.21 (br dd, *J* = 5.0, 4.8 Hz, 2 H, CH₂O), 4.15-4.18 (m, 1 H, H 1), 4.09 (s, 3 H, OCH₃), 4.02 (br d, *J* = 5.0, 4.8 Hz, 2 H, CH₂O), 3.97 (d, *J* = 11.5, 3.1 Hz, 1 H, CH₂Cl), 3.95 (s, 3 H, OCH₃), 3.92 (s, 3 H, OCH₃), 3.48 (dd, *J* = 11.5, 10.9 Hz, 1 H, CH₂Cl), 0.90 (s, 9 H, SiC(CH₃)₃), 0.10 (s, 6 H, Si(CH₃)₂); ¹³C NMR δ 160.3 (CO), 156.7 (C 2"), 154.0 (OCONH), 150.2 (C 5'), 148.5 (C 4"), 141.7 (C 3a), 140.6 (C 6'), 138.9 (C 7'), 133.8 (C

- 5), 132.3 (C 1'), 129.7 (C 2'), 129.6 (C 9a), 128.7 (C 6''), 127.5 (C 8), 125.6 (C 7a'), 125.0 (C 7), 123.6 (C 3a'), 123.1 (C 9), 122.4 (C 6, C 9b), 121.8 (C 5a), 115.8 (C 5''), 112.8 (C 4), 106.5 (C 3', C 3''), 97.7 (C 4'), 70.7 (CH₂O), 61.8 (CH₂O), 61.7 (CH₂O), 61.5 (OCH₃), 61.1 (OCH₃), 56.3 (OCH₃), 54.9 (C 2), 45.8 (CH₂Cl), 43.5 (C 1), 25.8 (SiC(CH₃)₃), 18.3 (SiC(CH₃)₃), -5.4 (Si(CH₃)₂); MS (FAB⁺) *m/z* 819 (MH⁺, 25%), 821 (MH⁺, 12); HRMS (FAB⁺) calcd for C₄₁H₄₈³⁵ClN₄O₁₀Si (MH⁺) *m/z* 819.2828, found 819.2804; calc. for C₄₁H₄₈³⁷ClN₄O₁₀Si (MH⁺) *m/z* 821.2799, found 821.2803; Anal. (C₄₁H₄₇ClN₄O₁₀Si) C, H, N.
- 10 2-(2-Hydroxyethoxy)-4-nitrobenzyl 1-(chloromethyl)-3-[(5,6,7-trimethoxy-1*H*-indol-2-yl)carbonyl]-2,3-dihydro-1*H*-benzo[*e*]indol-5-ylcarbamate (70). 1 M HCl (0.4 mL, 400 μmol) was added to a stirred solution of silyl ether 69 (157 mg, 192 μmol) in MeOH (5 mL) and the solution stirred at 20 °C for 1 h. The solvent was evaporated and the residue partitioned between EtOAc (50 mL) and water (50 mL). The organic fraction was washed with water (50 mL), brine (25 mL), dried, and the solvent evaporated. The residue was purified by chromatography, eluting with a gradient (20-50%) of EtOAc/light petroleum, to give 70 (119 mg, 88%) as a hygroscopic white solid, ¹H NMR δ 9.72 (s, 1 H, indole-NH), 8.80 (s, 1 H, OCONH), 7.86 (d, *J* = 8.5 Hz, 1 H, H 6), 7.79 (br d, *J* = 8.1 Hz, 1 H, H 5''), 7.67-7.73 (m, 2 H, H 9, H 3''), 7.47-7.53 (m, 3 H, H 4, H 8, H 6''), 7.37 (ddd, *J* = 8.5, 7.1, 0.8 Hz, 1 H, H 7), 6.97 (d, *J* = 2.2 Hz, 1 H, H 3'), 6.87 (s, 1 H, H 4'), 5.40 (s, 2 H, CH₂O), 4.73 (dd, *J* = 10.7, 1.6 Hz, 1 H, H 2), 4.59 (dd, *J* = 10.7, 8.7 Hz, 1 H, H 2), 4.21 (br dd, *J* = 4.6, 4.0 Hz, 2 H, CH₂O), 4.07-4.11 (m, 4 H, H 1, OCH₃), 4.00-4.04 (m, 2 H, CH₂O), 3.95 (s, 3 H, OCH₃), 3.92 (s, 3 H, OCH₃), 3.85 (d, *J* = 11.3, 3.0 Hz, 1 H, CH₂Cl), 3.39 (br s, 1 H, OH), 3.28 (dd, *J* = 11.3, 10.9 Hz, 1 H, CH₂Cl); ¹³C NMR δ 160.5 (CO), 157.2 (C 2''), 154.4 (OCONH), 150.2 (C 5'), 148.9 (C 4''), 141.4 (C 3a), 140.6 (C 6'), 138.9 (C 7'), 133.8 (C 5), 131.9 (C 1''), 130.3 (C 6''), 129.6 (C 2', C 9a), 127.4 (C 8), 125.8 (C 7a'), 125.0 (C 7), 123.6 (C 3a'), 123.0 (C 9), 122.5 (C 6, C 9b), 121.9 (C 5a), 115.9 (C 5''), 112.8 (C 4), 106.7 (C 3'), 106.6 (C 3''), 97.7 (C 4'), 70.7 (CH₂O), 62.0 (CH₂O), 61.5 (OCH₃), 61.1 (OCH₃), 60.9 (CH₂O), 56.3 (OCH₃), 55.1 (C 2), 45.6 (CH₂Cl), 43.3 (C 1); MS (FAB⁺) *m/z* 707 (MH⁺, 5%), 705 (MH⁺, 14); HRMS (FAB⁺) calcd for C₃₅H₃₄³⁵ClN₄O₁₀ (MH⁺) *m/z* 705.1964, found 705.1919; calc. for C₃₅H₃₄³⁷ClN₄O₁₀ (MH⁺) *m/z* 707.1934, found 707.1931; Anal. (C₃₅H₃₃ClN₄O₁₀) C, H, N.

Example 4B. Preparation of 2-(2-methoxyethoxy)-4-nitrobenzyl 1-(chloromethyl)-3-[(5,6,7-trimethoxy-1*H*-indol-2-yl)carbonyl]-2,3-dihydro-1*H*-benzo[*e*]indol-5-ylcarbamate (73).

- 5 **Methyl 2-(2-methoxyethoxy)-4-nitrobenzoate (71).** A mixture of methyl 2-hydroxy-4-nitrobenzoate (66) (1.0 g, 5.07 mmol) and K_2CO_3 (1.05 g, 7.61 mmol) in DMF (25 mL) was stirred at 20 °C for 30 min. A solution of 2-bromoethyl methyl ether (0.72 mL, 7.61 mmol) in DMF (3 mL) was added and the mixture was stirred at 100 °C for 4 h. The solvent was evaporated and the residue partitioned between EtOAc (100 mL) and water (100 mL). The
- 10 organic fraction was washed with water (2 × 50 mL), brine (50 mL), dried, and the solvent evaporated. The residue was purified by chromatography, eluting with 30% EtOAc/light petroleum, to give 71 (1.27 g, 98%) as a white solid, mp (EtOAc/light petroleum) 45-46 °C; 1H NMR δ 7.90 (d, J = 8.3 Hz, 1 H, H 6), 7.82-7.86 (m, 2 H, H 3, H 5), 4.28-4.30 (m, 2 H, CH_2O), 3.93 (s, 3 H, OCH_3), 3.82-3.87 (m, 2 H, CH_2O), 3.48 (s, 3 H, OCH_3); ^{13}C NMR δ
- 15 165.1 (CO_2), 158.6 (C 2), 150.6 (C 4), 132.1 (C 6), 126.4 (C 1), 115.2 (C 5), 108.4 (C 3), 70.5 (CH_2O), 69.4 (CH_2O), 59.4 (OCH_3), 52.5 (OCH_3); Anal. ($C_{11}H_{13}NO_6$) C, H, N.

- 2-[2-(Methoxy)ethoxy]-4-nitrobenzyl alcohol (72).** DIBALH (1 M in DCM, 16.4 mL, 16.4 mmol) was added dropwise to a stirred solution of ester 71 (1.27 g, 4.97 mmol) in
- 20 THF (100 mL) at 5 °C and the solution stirred at 5 °C for 1 h. The solution was poured into a solution of potassium sodium tartrate (1 M, 100 mL) and stirred vigorously for 30 min. The mixture was extracted with EtOAc (2 × 100 mL), the combined organic fraction washed with water (50 mL), brine (50 mL), dried and the solvent was evaporated. The residue was purified by chromatography, eluting with a gradient (30-50%) of EtOAc/light
- 25 petroleum, to give 72 (1.03 g, 91%) as a white solid, mp (EtOAc/light petroleum) 89-90.5 °C; 1H NMR δ 7.86 (dd, J = 8.2, 2.1 Hz, 1 H, H 5), 7.72 (d, J = 2.1 Hz, 1 H, H 3), 7.47 (d, J = 8.2 Hz, 1 H, H 6), 4.74 (br s, 2 H, CH_2O), 4.26-4.29 (m, 2 H, CH_2O), 3.78-3.80 (m, 2 H, CH_2O), 3.45 (s, 3 H, OCH_3), 3.10 (br s, 1 H, OH); ^{13}C NMR δ 156.8 (C 2), 148.2 (C 4), 137.5 (C 1), 128.6 (C 6), 116.5 (C 5), 107.0 (C 3), 70.5 (CH_2O), 68.4 (CH_2O), 61.1 (CH_2O),
- 30 59.1 (OCH_3); Anal. ($C_{10}H_{13}NO_5$) C, H, N.

2-(2-Methoxyethoxy)-4-nitrobenzyl 1-(chloromethyl)-3-[(5,6,7-trimethoxy-1*H*-indol-2-

yl)carbonyl]-2,3-dihydro-1H-benzo[e]indol-5-ylcarbamate (73). A solution of triphosgene (12 mg, 40 μ mol) in DCM (2 mL) was added dropwise to a stirred solution of amine 1 [G. J. Atwell, W. R. Wilson, W. A. Denny, *Bioorg. Med. Chem. Lett.*, 1997, 7, 1483] (53 mg, 114 μ mol) and Et₃N (32 μ L, 228 μ mol) in DCM (10 mL) and stirred at 20 °C for 2 h. A solution of 2-[2-(methoxy)ethoxy]-4-nitrobenzyl alcohol 72 (28 mg, 125 μ mol) in DCM (2 mL) was added, followed by nBu₂Sn(OAc)₂ (2 drops) and the solution stirred at 20 °C for 24 h. The solvent was evaporated and the residue purified by chromatography, eluting with 20% EtOAc/DCM, to give 73 (75 mg, 91%) as a tan gum, ¹H NMR δ 9.49 (s, 1 H, indole-NH), 8.19 (s, 1 H, OCONH), 7.92 (d, *J* = 8.5 Hz, 1 H, H 6), 7.80-7.82 (m, 1 H, H 5"), 7.78 (d, *J* = 8.3 Hz, 1 H, H 9), 7.71 (d, *J* = 1.8 Hz, 1 H, H 3"), 7.56 (ddd, *J* = 8.3, 7.1, 0.8 Hz, 1 H, H 8), 7.49-7.54 (m, 1 H, H 6"), 7.45 (ddd, *J* = 8.5, 7.1, 0.8 Hz, 1 H, H 7), 7.27 (br s, 1 H, H 4), 7.00 (d, *J* = 2.3 Hz, 1 H, H 3'), 6.87 (s, 1 H, H 4'), 5.39 (s, 2 H, CH₂O), 4.79 (dd, *J* = 10.7, 1.7 Hz, 1 H, H 2), 4.66 (dd, *J* = 10.7, 8.7 Hz, 1 H, H 2), 4.23 (dd, *J* = 4.6, 4.4 Hz, 2 H, CH₂O), 4.15-4.20 (m, 1 H, H 1), 4.08 (s, 3 H, OCH₃), 3.94-3.98 (m, 4 H, OCH₃, CH₂Cl), 3.91 (s, 3 H, OCH₃), 3.80 (dd, *J* = 4.6, 4.4 Hz, 2 H, CH₂O), 3.47 (d, *J* = 10.9 Hz, 1 H, CH₂Cl), 3.44 (s, 3 H, OCH₃); ¹³C NMR δ 160.3 (CO), 156.5 (C 2"), 154.0 (OCONH), 150.2 (C 5'), 148.5 (C 4"), 141.7 (C 3a), 140.6 (C 6'), 138.9 (C 7'), 133.9 (C 5), 132.4 (C 1"), 129.7 (C 2'), 129.6 (C 9a), 128.7 (C 6"), 127.5 (C 8), 125.6 (C 7a'), 125.0 (C 7), 123.6 (C 3a'), 123.1 (C 9), 122.5 (C 6, C 9b), 121.8 (C 5a), 116.0 (C 5"), 112.7 (C 4), 106.5 (C 3'), 106.3 (C 3"), 97.7 (C 4'), 70.7 (CH₂O), 68.5 (CH₂O), 61.9 (CH₂O), 61.5 (OCH₃), 61.1 (OCH₃), 59.3 (OCH₃), 56.3 (OCH₃), 54.9 (C 2), 45.9 (CH₂Cl), 43.1 (C 1); MS (FAB⁺) *m/z* 721 (MH⁺, 1.5%), 719 (MH⁺, 3.5); HRMS (FAB⁺) calc. for C₃₆H₃₆³⁷ClN₄O₁₀ (MH⁺) *m/z* 721.2091, found 721.2131; calc. for C₃₆H₃₆³⁵ClN₄O₁₀ (MH⁺) *m/z* 719.2120, found 719.2133; Anal. (C₃₆H₃₅ClN₄O₁₀) C, H, N.

Example 4C. Preparation of 1-[2-(3-hydroxypropoxy)-4-nitrophenyl]ethyl 4-[bis(2-chloroethyl)amino]phenylcarbamate (78).

Methyl 4-nitro-2-[3-(tetrahydro-2H-pyran-2-yloxy)propoxy]benzoate (74). A mixture of methyl 4-nitrosalicylate (66) (2.3 g, 11.7 mmol) and K₂CO₃ (2.42 g, 17.5 mmol) in DMF (25 mL) was stirred at 20 °C for 20 min. A solution of 3-iodopropyl tetrahydropyranyl ether (4.7 g, 17.5 mmol) in DMF (5 mL) was added and the mixture stirred at 100 °C for 2 h. The mixture was poured into water, extracted with EtOAc (3 \times 100 mL), the combined

organic extracts washed with water (2 × 50 mL), brine (50 mL), dried and the solvent evaporated. The residue was purified by chromatography, eluting with 20% EtOAc/light petroleum, to give 74 (3.66 g, 92 %) as a colourless oil, ¹H NMR δ 7.89 (d, *J* = 8.5 Hz, 1 H, H 6), 7.80-7.84 (m, 2 H, H 3, H 5), 4.60-4.62 (m, 1 H, OCHO), 4.27 (t, *J* = 6.2 Hz, 2 H, CH₂O), 3.95-4.00 (m, 1 H CH₂O), 3.94 (s, 3 H, OCH₃), 3.79-3.86 (m, 1 H, CH₂O), 3.59-3.66 (m, 1 H, CH₂O), 3.47-3.52 (m, 1 H, CH₂O), 2.13-2.17 (m, 2 H, CH₂), 1.78-1.84 (m, 1 H, CH₂), 1.68-1.75 (m, 1 H, CH₂), 1.47-1.62 (m, 4 H, 2 CH₂); ¹³C NMR δ 164.5, 158.6, 150.7, 132.0, 126.2, 114.8, 107.9, 99.0, 66.5, 63.4, 62.4, 52.5, 30.6, 29.3, 25.4, 19.6; MS *m/z* 339 (M⁺, 2%), 322 (12), 239 (20), 222(40), 85 (100); HRMS calc. for C₁₆H₂₁NO₇ (M⁺) *m/z* 339.1318, found 339.1317.

{4-Nitro-2-[3-(tetrahydro-2*H*-pyran-2-yloxy)propoxy]phenyl}methanol (75). DIBALH (1 M in DCM, 34 mL, 34 mmol) was added dropwise to a solution of 74 (3.46 g, 10.2 mmol) in THF (100 mL) at 5 °C and the solution stirred at 5 °C for 1 h. The solution was poured into a solution of sodium potassium tartrate (1 M, 100 mL) and stirred for 30 min. The mixture was extracted with EtOAc (3 × 100 mL), the combined organic fraction washed with water (100 mL), brine (50 mL), dried, and the solvent evaporated. The residue was purified by chromatography, eluting with 50%EtOAc/light petroleum, to give 75 (3.11 g, 98 %) as a pale yellow solid, mp (EtOAc/light petroleum) 64-65.5 °C; ¹H NMR δ 7.84 (dd, *J* = 8.2, 2.1 Hz, 1 H, H 5), 7.72 (d, *J* = 2.1 Hz, 1 H, H 3), 7.50 (d, *J* = 8.2 Hz, 1 H, H 6), 4.74 (dd, *J* = 14.8, 4.2 Hz, 2 H, CH₂O), 4.58-4.61 (m, 1 H, OCHO), 4.24 (t, *J* = 6.1 Hz, 2 H, CH₂O), 3.96 (dt, *J* = 10.0, 5.8 Hz, 1 H, CH₂O), 3.80-3.86 (m, 1 H, CH₂O), 3.62 (dt, 10.0, 5.8 Hz, 1 H, CH₂O), 3.46-3.51 (m, 1 H, CH₂), 2.30 (br s, 1 H, OH), 2.08-2.11 (m, 2 H, CH₂), 1.79-1.85 (m, 1 H, CH₂), 1.69-1.77 (m, 1 H, CH₂), 1.48-1.62 (m, 4 H, 2 × CH₂); ¹³C NMR δ 156.5, 148.2, 136.8, 128.1, 115.9, 105.8, 99.3, 65.9, 63.9, 62.8, 60.8, 30.6, 29.3, 25.3, 19.7; MS (CI, NH₃) *m/z* 312 (MH⁺, 0.5%), 294 (1), 245 (15), 227(30), 85 (100); HRMS (CI, NH₃) calc. for C₁₅H₂₂NO₆ (MH⁺) *m/z* 312.1447, found 312.1438. Anal. (C₁₅H₂₁NO₆) C, H, N.

4-Nitro-2-[3-(tetrahydro-2*H*-pyran-2-yloxy)propoxy]benzyl 4-[bis(2-hydroxyethyl)amino]phenylcarbamate (76). Pyridine (135 μL, 1.67 mmol) was added dropwise to a stirred solution of alcohol 75 (521 mg, 1.67 mmol) and triphosgene (174 mg,

0.59 mmol) in THF (20 mL) at 5 °C and the suspension stirred at 5 °C for 1 h. A solution of *N*¹,*N*¹-bis(2-hydroxyethyl)-1,4-benzenediamine **57** [prepared by catalytic hydrogenation of *N,N*-bis-(2-hydroxyethyl) 4-nitroaniline (360 mg, 1.84 mmol) with Pd/C under H₂ (60 psi) in EtOH] in THF (10 mL) and DMF (10 mL) was added and the mixture stirred at 20 °C for 16 h. The solvent was evaporated and the residue partitioned between EtOAc/water (100 mL). The aqueous fraction was extracted with EtOAc (2 × 50 mL) and the combined organic fraction washed with brine (50 mL), dried and the solvent evaporated. The residue was purified by chromatography, eluting with 80-100% EtOAc/light petroleum to give **76** (220 mg, 25 %) as a colourless oil, ¹H NMR [(CD₃)₂SO] δ 9.42 (s, 1 H, OCONH), 7.88 (dd, *J* = 8.3, 2.0 Hz, 1 H, H 5'), 7.79 (d, *J* = 2.0 Hz, 1 H, H 3'), 7.61 (br d, *J* = 8.3 Hz, 1 H, H 6'), 7.72 (br d, *J* = 9.1 Hz, 2 H, H 2, H 6), 6.61 d, *J* = 9.1 Hz, 2 H, H 3, H 5), 5.19 (s, 2 H, CH₂O), 4.73 (t, *J* = 5.4 Hz, 1 H, CH₂O), 4.54-4.58 (m, 1 H, OCHO), 4.23 (t, *J* = 6.1 Hz, 2 H, CH₂O), 3.80 (dt, *J* = 9.9, 6.4 Hz, 1 H, CH₂O), 3.67-3.71 (m, 1 H, CH₂O), 3.55 (t, *J* = 5.4 Hz, 1 H, CH₂O), 3.50 (t, *J* = 6.0 Hz, 4 H, 2 × CH₂O), 3.32-3.35 (m, 4 H, 2 × CH₂N), 1.99-2.06 (m, 2 H, CH₂), 1.67-1.73 (m, 1 H, CH₂), 1.56-1.62 (m, 1 H, CH₂), 1.40-1.49 (m, 4 H, 2 × CH₂); ¹³C NMR [(CD₃)₂SO] δ 156.2, 153.2, 148.1, 144.1, 132.9, 128.6, 127.4, 120.2 (2), 115.4, 111.4 (2), 106.0, 98.0, 65.7, 63.0, 61.3, 60.1, 58.1 (2), 53.4 (2), 30.2, 28.8, 25.0, 19.1; MS (FAB⁺) *m/z* 533 (M⁺, 20 %); HRMS (FAB⁺) calc. for C₂₆H₃₅N₃O₉ (M⁺) *m/z* 533.2373, found 533.2365.

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2-(3-Hydroxypropoxy)-4-nitrobenzyl 4-[bis(2-chloroethyl)amino]phenylcarbamate (78). Methanesulphonyl chloride (85 μL, 1.1 mmol) was added dropwise to a stirred solution of **76** (195 mg, 0.36 mmol) in pyridine (10 mL) at 20 °C and the solution stirred for 1 h. The solvent was evaporated and the residue partitioned between DCM/water (100 mL). The aqueous fraction was extracted with DCM (2 × 50 mL) and the combined organic fraction washed with brine (50 mL), dried and the solvent evaporated. The residue was dissolved in DMF (10 mL), LiCl (93 mg, 2.2 mmol) added, and the mixture stirred at 80 °C for 3 h. The solvent was evaporated and the residue partitioned between EtOAc (100 mL) and water (100 mL). The aqueous fraction was extracted with EtOAc (2 × 50 mL). The combined organic fraction was washed with brine (50 mL), dried, and the solvent evaporated. The residue was purified by chromatography, eluting with 25% EtOAc/light petroleum, to give (i) 4-nitro-2-[3-(tetrahydro-2*H*-pyran-2-yloxy)propoxy]benzyl 4-[bis(2-

30

chloroethyl)amino]phenylcarbamate (77) (46 mg, 22 %) as a colourless oil, ^1H NMR δ 7.82 (dd, $J = 8.3, 2.1$ Hz, 1 H, H 5'), 7.73 (d, $J = 2.1$ Hz, 1 H, H 3'), 7.50 (br d, $J = 8.3$ Hz, 1 H, H 6'), 7.26-7.29 (m, 3 H, OCONH, H 2, H 6), 6.65 (ddd, $J = 9.1, 3.4, 1.9$ Hz, 2 H, H 3, H 5), 5.25 (s, 2 H, CH_2O), 4.67 (br s, 1 H, OCHO), 4.20-4.27 (m, 2 H, CH_2O), 4.01 (dt, $J = 9.7, 6.1$ Hz, 1 H, CH_2O), 3.65-3.75 (m, 6 H, CH_2O , $2 \times \text{CH}_2\text{N}$), 3.58-3.63 (m, 4 H, $2 \times \text{CH}_2\text{Cl}$), 3.44-3.50 (m, 1 H, CH_2O), 2.11-2.15 (m, 2 H, CH_2), 1.66-1.72 (m, 1 H, CH_2), 1.55-1.62 (m, 1 H, CH_2), 1.40-1.50 (m, 4 H, $2 \times \text{CH}_2$); ^{13}C NMR δ 157.2, 153.6, 148.8, 142.6, 132.0, 129.8, 128.7, 121.3 (2), 115.5, 112.8 (2), 105.9, 98.4, 65.3, 63.0, 61.7 (2), 53.7 (2), 40.5 (2), 30.5, 29.3, 25.4, 19.0; MS (FAB $^+$) m/z 569 (M^+ , 3%); HRMS (FAB $^+$) calc for $\text{C}_{26}\text{H}_{33}^{35}\text{Cl}_2\text{N}_3\text{O}_7$ (M^+) m/z 569.1696, found 569.1689; calc. for $\text{C}_{26}\text{H}_{33}^{35}\text{Cl}^{37}\text{ClN}_3\text{O}_7$ (M^+) m/z 571.1666, found 569.1680; calc. for $\text{C}_{26}\text{H}_{33}^{37}\text{Cl}_2\text{N}_3\text{O}_7$ (M^+) m/z 573.1637, found 569.1654.

Further elution gave 78 (99 mg, 57 %) as a white powder, mp (DCM/pet. ether) 104-105 $^\circ\text{C}$; ^1H δ 7.84 (dd, $J = 8.3, 2.0$ Hz, 1 H, H 5'), 7.74 (d, $J = 2.0$ Hz, 1 H, H 3'), 7.51 (br d, $J = 8.3$ Hz, 1 H, H 6'), 7.24 (br d, $J = 9.0$ Hz, 2 H, H 2, H 6), 6.87 (br s, 1 H, OCONH), 6.64 (d, $J = 9.0$ Hz, 2 H, H 3, H 5), 5.27 (s, 2 H, CH_2O), 4.27 (t, $J = 5.8$ Hz, 2 H, CH_2O), 3.89 (t, $J = 5.7$ Hz, 2 H, CH_2O), 3.67-3.72 (m, 4 H, $2 \times \text{CH}_2\text{N}$), 3.58-3.64 (m, 4 H, $2 \times \text{CH}_2\text{Cl}$), 2.08-2.12 (m, 2 H, CH_2); ^{13}C NMR δ 156.8, 153.4, 148.7, 145.6, 132.0, 129.4, 128.2, 123.4 (2), 115.6, 112.7 (2), 106.1, 66.6, 61.5, 59.9, 53.6 (2), 40.5 (2), 31.6; Anal. ($\text{C}_{21}\text{H}_{22}\text{Cl}_2\text{N}_3\text{O}_6$) C, H, N, Cl.

Example 4D. Preparation of 2-(3-hydroxypropoxy)-4-nitrobenzyl 3-(chloromethyl)-1-[(5,6,7-trimethoxy-1H-indol-2-yl)carbonyl]-2,3-dihydro-1H-indol-6-ylcarbamate (80). Pyridine (35 μL , 0.44 mmol) was added dropwise to a stirred solution of alcohol 75 (45 mg, 0.25 mmol) and triphosgene (45 mg, 0.15 mmol) in THF (10 mL) at 5 $^\circ\text{C}$ and the suspension stirred at 5 $^\circ\text{C}$ for 1 h. A solution of 3-(chloromethyl)-1-[(5,6,7-trimethoxy-1H-indol-2-yl)carbonyl]-2,3-dihydro-1H-indol-6-ylamine (33) [M. Tercel and W. A. Denny. *J. Chem. Soc. Perkin Trans. I*, 1998, 509] (199 mg, 0.48 mmol) in THF (10 mL) was added and the mixture stirred at 20 $^\circ\text{C}$ for 16 h. The suspension was filtered and the solvent evaporated. The residue was purified by chromatography, eluting with 40% EtOAc/DCM, to give (i) 4-nitro-2-[3-(tetrahydro-2H-pyran-2-yloxy)propoxy]benzyl 3-(chloromethyl)-1-[(5,6,7-trimethoxy-1H-indol-2-yl)carbonyl]-2,3-dihydro-1H-indol-6-ylcarbamate 79 (116

mg, 35%) as an oil, ^1H NMR δ 9.54 (s, 1 H, NH), 8.24 (s, 1 H, H 7), 7.84 (s, 1 H, H 4), 7.77 (dd, $J = 8.3, 2.0$ Hz, 1 H, H 5"), 7.71 d, $J = 2.0$ Hz, 1 H, H 3"), 7.52 (br s, 1 H, OCONH), 7.48 (d, $J = 8.3$ Hz, 1 H, H 6"), 7.22 (d, $J = 8.3$ Hz, 1 H, H 4), 6.93 (d, $J = 2.2$ Hz, 1 H, H 3'), 6.85 (s, 1 H, H 4'), 5.27 (s, 2 H, CH_2O), 4.69 (br s, 1 H, OCHO), 4.62 (dd, $J = 10.6, 9.4$ Hz, 1 H, H 2), 4.45 (dd, $J = 10.6, 3.8$ Hz, 1 H, H 2), 4.20-4.24 (m, 2 H, CH_2O), 4.08 (s, 3 H, OCH_3), 3.94-3.98 (m, 1 H, CH_2O), 3.93 (s, 3 H, OCH_3), 3.91 (s, 3 H, OCH_3), 3.71-3.82 (m, 3 H, H 3, CH_2O , CH_2Cl), 3.49-3.61 (m, 3 H, CH_2Cl , CH_2O), 2.10-2.15 (m, 2 H, CH_2), 1.65-1.78 (m, 2 H, CH_2), 1.45-1.60 (m, 4 H, $2 \times \text{CH}_2$); ^{13}C NMR δ 160.3, 157.2, 153.3, 150.2, 148.8, 144.1, 140.5, 138.9, 138.8, 131.8, 129.6, 129.5, 125.9, 125.5, 124.5, 123.6, 115.5, 114.6, 108.7, 107.9, 105.9, 98.4, 97.6, 67.6, 65.4, 63.0, 61.8, 61.4, 61.1, 56.2, 54.7, 46.9, 43.2, 30.6, 29.2, 25.4, 19.0; MS (FAB $^+$) m/z 752 (M^+ , 8%), 669 (20), 234 (30); HRMS (FAB $^+$) calc. for $\text{C}_{37}\text{H}_{41}^{35}\text{ClN}_4\text{O}_{11}$ (M^+) m/z 752.2460, found 752.2455; calc. for $\text{C}_{37}\text{H}_{41}^{37}\text{ClN}_4\text{O}_{11}$ (M^+) m/z 754.2431, found 754.2424.

15 Further elution gave **80** (44 mg, 15%) as a tan solid, mp (EtOAc/light petroleum) 166-168 $^\circ\text{C}$; ^1H NMR δ 9.47 (s, 1 H, indole-NH), 8.24 (d, $J = 1.8$ Hz, 1 H, H 7), 7.79 (dd, $J = 8.3, 2.1$ Hz, 1 H, H 5"), 7.71 (d, $J = 2.1$ Hz, 1 H, H 3"), 7.48 (d, $J = 8.3$ Hz, 1 H, H 6"), 7.42 (br s, 1 H, OCONH), 7.33 (s, 1 H, H 5), 7.21 (d, $J = 8.2$ Hz, 1 H, H 4), 6.93 (d, $J = 2.3$ Hz, 1 H, H 3'), 6.85 (s, 1 H, H 4'), 5.29 (s, 2 H, CH_2O), 4.62 (dd, $J = 10.8, 9.8$ Hz, 1 H, H 2), 4.46 (dd, $J = 10.8, 4.5$ Hz, 1 H, H 2), 4.25 (t, $J = 5.8$ Hz, 2 H, CH_2O), 4.07 (s, 3 H, OCH_3), 3.94 (s, 3 H, OCH_3), 3.88-3.93 (m, 5 H, OCH_3 , CH_2O), 3.79-3.85 (m, 2 H, H 3, CH_2Cl), 3.55 (dd, $J = 12.1, 10.3$ Hz, 1 H, CH_2Cl), 2.28 (br t, $J = 4.9$ Hz, 1 H, OH), 2.09-2.14 (m, 2 H, CH_2); ^{13}C NMR δ 160.5, 156.8, 153.1, 150.1, 148.5, 144.0, 140.5, 138.7, 138.6, 131.7, 130.8, 129.5, 129.3, 126.0, 125.8, 124.5, 123.5, 115.5, 114.6, 108.5, 106.7, 106.0, 66.0, 61.7, 61.5, 61.1, 59.4, 56.1, 54.7, 46.9, 43.2, 31.6; MS (FAB $^+$) m/z 671 (MH^+ , 1%), 669 (MH^+ , 3%), 391 (15), 149 (100); HRMS (FAB $^+$) calc. for $\text{C}_{32}\text{H}_{34}^{35}\text{ClN}_4\text{O}_{10}$ (MH^+) m/z 669.1964, found 669.1921; calc. for $\text{C}_{32}\text{H}_{34}^{37}\text{ClN}_4\text{O}_{10}$ (MH^+) m/z 671.1875; Anal. ($\text{C}_{32}\text{H}_{33}\text{ClN}_4\text{O}_{10} \cdot \frac{1}{2}\text{H}_2\text{O}$) C, H, N.

30 Compound **80** was also prepared by treating a solution of **79** (96 mg, 0.13 mmol) in MeOH (5 mL) with 0.1 M HCl (2 mL) and stirring at 20 $^\circ\text{C}$ for 16 h. The solvent was evaporated and the residue partitioned between DCM (50 mL) and water (50 mL). The organic fraction

was washed with water (10 mL), brine (10 mL), dried, and the solvent evaporated. The residue was purified by chromatography, eluting with 40% EtOAc/DCM, to give **80** (69 mg, 79%) as a tan solid, spectroscopically identical with the sample prepared above.

5 **Example 4E. Preparation of 2-(3-hydroxypropoxy)-4-nitrobenzyl 1-(chloromethyl)-3-[(5,6,7-trimethoxy-1H-indol-2-yl)carbonyl]-2,3-dihydro-1H-benzo[e]indol-5-ylcarbamate (84).**

Methyl 2-(3-{{*tert*-butyl(dimethyl)silyl}oxy}propoxy)-4-nitrobenzoate (**81**). A mixture of methyl 2-hydroxy-4-nitrobenzoate (**66**) (1.82 g, 9.23 mmol) and K₂CO₃ (1.91 g, 13.85 mmol) in DMF (30 mL) was stirred at 20 °C for 30 min. A solution of 3-bromopropyl *tert*-butyl(dimethyl)silyl ether (3.50 g, 13.85 mmol) in DMF (10 mL) was added and the mixture stirred at 100 °C for 3 h. The mixture was poured into water (300 mL), extracted with EtOAc (3 × 100 mL) and the combined organic extract washed with water (2 × 100 mL), brine (50 mL), dried and the solvent evaporated. The residue was purified by chromatography, eluting with 10% EtOAc/light petroleum, to give **81** (3.19 g, 93%) as a pale yellow solid, mp (EtOAc) 36.5-37 °C; ¹H NMR δ 7.88 (d, *J* = 8.9 Hz, 1 H, H 6), 7.80-7.84 (m, 2 H, H 3, H 5), 4.24 (t, *J* = 6.0 Hz, 2 H, CH₂O), 3.92 (s, 3 H, OCH₃), 3.85 (t, *J* = 5.9 Hz, 2 H, CH₂O), 2.04-2.09 (m, 2 H, CH₂), 0.88 (s, 9 H, OSi(CH₃)₃), 0.04 (s, 6 H, OSi(CH₃)₂); ¹³C NMR δ 165.4 (CO₂), 158.7 (C 2), 150.7 (C 4), 132.0 (C 6), 126.1 (C 1), 114.8 (C 5), 107.7 (C 3), 64.0 (CH₂O), 59.0 (CH₂O), 52.5 (OCH₃), 32.0 (CH₂), 25.9 (SiC(CH₃)₃), 18.3 (SiC(CH₃)₃), -5.5 (Si(CH₃)₂); Anal. (C₁₇H₂₇NO₆Si) C, H, N.

[2-(3-{{*tert*-Butyl(dimethyl)silyl}oxy}propoxy)-4-nitrophenyl]methanol (**82**). DIBALH (1 M in DCM, 16.5 mL, 16.5 mmol) was added to a stirred solution of ester **81** (1.85 g, 5.0 mmol) in THF (100 mL) at 5 °C and the solution stirred at 5 °C for 1 h. The solution was poured into a solution of potassium sodium tartrate (1 M, 100 mL) and the mixture stirred vigorously for 20 min. The mixture was extracted with EtOAc (3 × 100 mL), the combined organic fraction washed with water (100 mL), brine (50 mL), dried, and the solvent evaporated. The residue was purified by chromatography, eluting with 20% EtOAc/light petroleum, to give **82** (1.64 g, 94%) as a pale yellow solid, mp (EtOAc/light petroleum) 48-49 °C; ¹H NMR δ 7.84 (dd, *J* = 8.3, 2.1 Hz, 1 H, H 5), 7.71 (d, *J* = 2.1 Hz, 1 H, H 3), 7.51 (d, *J* = 8.3 Hz, 1 H, H 6), 4.76 (d, *J* = 6.3 Hz, 2 H, CH₂O), 4.21 (t, *J* = 6.1 Hz, 2 H, CH₂O),

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3.82 (t, $J = 5.9$ Hz, 2 H, CH_2OSi), 2.40 (t, $J = 6.3$ Hz, 1 H, OH), 2.02-2.08 (m, 2 H, CH_2), 0.89 (s, 9 H, $\text{OSiC}(\text{CH}_3)_3$), 0.06 (s, 6 H, $\text{OSi}(\text{CH}_3)_2$); ^{13}C NMR δ 156.5 (C 2), 148.2 (C 4), 136.7 (C 1), 127.8 (C 6), 115.9 (C 5), 105.8 (C 3), 65.5 (CH_2O), 60.8 (CH_2O), 59.3 (CH_2O), 32.0 (CH_2), 25.9 ($\text{OSiC}(\text{CH}_3)_3$), 18.3 ($\text{OSiC}(\text{CH}_3)_3$), -5.4 ($\text{OSi}(\text{CH}_3)_2$); Anal. ($\text{C}_{16}\text{H}_{27}\text{NO}_5\text{Si}$)

5 C, H, N.

2-(3-[[*tert*-Butyl(dimethyl)silyl]oxy]propoxy)-4-nitrobenzyl 1-(chloromethyl)-3-[(5,6,7-trimethoxy-1*H*-indol-2-yl)carbonyl]-2,3-dihydro-1*H*-benzo[*e*]indol-5-ylcarbamate

- (83). A solution of triphosgene (15 mg, 51 μmol) in DCM (2 mL) was added dropwise to a stirred solution of amine 1 [G. J. Atwell, W. R. Wilson, W. A. Denny, *Bioorg. Med. Chem. Lett.*, 1997, 7, 1483] (60 mg, 129 μmol) and Et_3N (40 μL , 289 μmol) in DCM (10 mL) and stirred at 20 °C for 2 h. A solution of alcohol 82 (54 mg, 159 μmol) in DCM (2 mL) was added, followed by $n\text{Bu}_2\text{Sn}(\text{OAc})_2$ (2 drops) and the solution stirred at 20 °C for 24 h. The solvent was evaporated and the residue purified by chromatography, eluting with a gradient (0-10%) MeOH/EtOAc, to give 83 (72 mg, 67%) as a yellow solid mp (MeOH) 149-151 °C; ^1H NMR δ 9.42 (s, 1 H, indole-NH), 8.96 (s, 1 H, OCONH), 7.91 (d, $J = 8.4$ Hz, 1 H, H 6), 7.78-7.85 (m, 2 H, H 9, H 5"), 7.75 (d, $J = 1.7$ Hz, 1 H, H 3"), 7.53-7.59 (m, 2 H, H 8, H 6"), 7.47 (ddd, $J = 8.4, 7.4, 0.8$ Hz, 1 H, H 7), 7.08 (br s, 1 H, H 4), 7.02 (d, $J = 2.2$ Hz, 1 H, H 3'), 6.89 (s, 1 H, H 4'), 5.38 (s, 2 H, CH_2O), 4.82 (dd, $J = 10.7, 1.7$ Hz, 1 H, H 2), 4.69 (dd, $J = 10.7, 8.7$ Hz, 1 H, H 2), 4.21 (t, $J = 6.0$ Hz, 2 H, CH_2O), 4.17-4.20 (m, 1 H, CH_2Cl), 4.09 (s, 3 H, OCH_3), 3.99 (dd, $J = 11.3, 2.9$ Hz, 1 H, H 1), 3.95 (s, 3 H, OCH_3), 3.92 (s, 3 H, OCH_3), 3.83 (t, $J = 5.9$ Hz, 2 H, CH_2O), 3.49 (t, $J = 11.0$ Hz, 1 H, CH_2Cl), 2.02-2.08 (m, 2 H, CH_2), 0.88 (s, 9 H, $\text{OSiC}(\text{CH}_3)_3$), 0.04 (s, 6 H, $\text{OSi}(\text{CH}_3)_2$); MS (FAB $^+$) m/z 833 (MH^+ , 25%), 835 (MH^+ , 12), 775 (5), 599 (5); HRMS (FAB $^+$) calc. for $\text{C}_{42}\text{H}_{50}^{35}\text{ClN}_4\text{O}_{10}\text{Si}$ (MH^+) m/z 833.2985, found 833.3008; calc. for $\text{C}_{42}\text{H}_{50}^{37}\text{ClN}_4\text{O}_{10}\text{Si}$ (MH^+) m/z 835.2955, found 835.2982; Anal. ($\text{C}_{42}\text{H}_{49}\text{ClN}_4\text{O}_{10}\text{Si}$) C, H, N.

- 2-(3-Hydroxypropoxy)-4-nitrobenzyl 1-(chloromethyl)-3-[(5,6,7-trimethoxy-1*H*-indol-2-yl)carbonyl]-2,3-dihydro-1*H*-benzo[*e*]indol-5-ylcarbamate (84).** 1 M HCl (0.2 mL, 200 μmol) was added to a stirred solution of silyl ether 83 (64 mg, 77 μmol) in MeOH (5 mL) and the solution stirred at 20 °C for 30 min. The solvent was evaporated and the residue dissolved in EtOAc (50 mL), washed with water (2 \times 50 mL), brine (25 mL), dried

and the solvent evaporated. The residue was purified by chromatography, eluting with a gradient (50-100%) of EtOAc/light petroleum, to give **84** (52 mg, 94%) as a tan solid, mp (EtOAc) 122-126 °C; ¹H NMR δ 9.51 (s, 1 H, indole-NH), 8.90 (s, 1 H, OCONH), 7.92 (d, *J* = 8.5 Hz, 1 H, H 6), 7.80 (d, *J* = 8.2 Hz, 1 H, H 5"), 7.77 (d, *J* = 8.3 Hz, 1 H, H 9), 7.73 (d, *J* = 1.8 Hz, 1 H, H 3"), 7.50-7.57 (m, 2 H, H 8, H 6"), 7.40-7.46 (m, 2 H, H 4, H 7), 6.99 (d, *J* = 2.2 Hz, 1 H, H 3'), 6.87 (s, 1 H, H 4'), 5.37 (d, *J* = 13.1 Hz, 1 H, CH₂O), 5.32 (d, *J* = 13.1 Hz, 1 H, CH₂O), 4.77 (dd, *J* = 10.8, 1.6 Hz, 1 H, H 2), 4.64 (dd, *J* = 10.8, 8.6 Hz, 1 H, H 2), 4.27 (t, *J* = 5.7 Hz, 2 H, CH₂O), 4.11-4.18 (m, 1 H, CH₂Cl), 4.09 (s, 3 H, OCH₃), 3.96 (s, 3 H, OCH₃), 3.91-3.95 (m, 3 H, H 1, CH₂O), 3.90 (s, 3 H, OCH₃), 3.44 (t, *J* = 10.9 Hz, 1 H, CH₂Cl), 2.75 (br s, 1 H, OH), 2.12-2.18 (m, 2 H, CH₂); ¹³C NMR δ 160.4 (CO), 157.2 (C 2"), 153.8 (OCONH), 150.2 (C 5'), 148.9 (C 4"), 141.6 (C 3a), 140.6 (C 6'), 138.9 (C 7"), 134.0 (C 5), 131.6 (C 1"), 130.1 (C 6"), 129.7 (C 2'), 129.6 (C 9a), 127.5 (C 8), 125.7 (C 7a'), 125.0 (C 7), 123.6 (C 3a'), 123.1 (C 9), 122.4 (C 6, C 9b), 121.6 (C 5a), 115.7 (C 5"), 112.2 (C 4), 106.6 (C 3'), 106.1 (C 3"), 97.7 (C 4'), 66.8 (CH₂O), 62.2 (CH₂O), 61.5 (OCH₃), 61.1 (OCH₃), 60.1 (CH₂O), 56.3 (OCH₃), 55.0 (C 2), 45.8 (CH₂Cl), 43.4 (C 1), 31.6 (CH₂); MS (FAB⁺) *m/z* 721 (MH⁺, 2%), 719 (MH⁺, 4); HRMS (FAB⁺) calc. for C₃₆H₃₅³⁵CIN₄O₁₀ (MH⁺) *m/z* 719.2120, found 719.2107; calc. for C₃₆H₃₅³⁷CIN₄O₁₀ (MH⁺) *m/z* 721.2091, found 721.2093; Anal. (C₃₆H₃₅CIN₄O₁₀) C, H, N.

20 Example 4F. Preparation of 2-(3-hydroxypropoxy)-4-nitrobenzyl doxorubicin carbamate (87).

4-Nitrophenyl 4-nitro-2-[3-(tetrahydro-2H-pyran-2-yloxy)propoxy]benzyl carbonate (85). A solution of 4-nitrophenylchloroformate (0.43 g, 2.1 mmol) in THF (10 mL) was added dropwise to a stirred solution of alcohol **75** (0.44 g, 1.4 mmol) and DIEA (0.49 mL, 2.8 mmol) in THF (40 mL) and the mixture stirred at 20 °C for 48 h. The solution was evaporated and the residue partitioned between EtOAc (100 mL) and water (100 mL). The organic fraction was washed with water (3 × 50 mL), brine (50 mL), dried, and the solvent evaporated. The residue was purified by chromatography, eluting with a gradient (10-50%) EtOAc/light petroleum, to give (i) starting material (176 mg, 40%); and (ii) **85** (0.38 g, 56%) as a pale yellow oil, ¹H NMR δ 8.30 (ddd, *J* = 9.2, 3.1, 2.1 Hz, 2 H, H 3, H 5), 7.87 (dd, *J* = 8.4, 2.1 Hz, 1 H, H 5'), 7.79 (d, *J* = 2.1 Hz, 1 H, H 3'), 7.57 (d, *J* = 8.4 Hz, 1 H, H 6'), 7.41 (ddd, *J* = 9.2, 3.1, 2.1 Hz, 2 H, H 2, H 6), 5.42 (s, 2 H, CH₂O), 4.58-4.61 (m, 1 H,

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OCHO), 4.28 (t, $J = 6.3$ Hz, 2 H, CH₂O), 3.96 (dt, $J = 10.0, 6.0$ Hz, 1 H, CH₂O), 3.78-3.83 (m, 1 H, CH₂O), 3.59 (dt, $J = 10.0, 6.0$ Hz, 1 H, CH₂O), 3.45-3.52 (m, 1 H, CH₂O), 2.13-2.18 (m, 2 H, CH₂O), 1.79-1.86 (m, 1 H, CH₂), 1.67-1.76 (m, 1 H, CH₂), 1.48-1.60 (m, 4 H, 2 × CH₂); ¹³C NMR δ 157.0 (C 1), 155.4 (C 2'), 153.4 (OCONH), 149.2 (C 4'), 145.5 (C 4), 129.9 (C 1), 129.2 (C 6'), 125.3 (C 3, C 5), 121.7 (C 2, C 6), 115.6 (C 5'), 106.3 (C 6'), 99.1 (OCO), 66.1 (CH₂O), 65.3 (CH₂O), 63.5 (CH₂O), 60.4 (CH₂O), 30.6 (CH₂), 29.4 (CH₂), 25.4 (CH₂), 19.7 (CH₂); MS m/z 476 (M⁺, 2%), 459 (5), 392 (2), 210(30), 85 (100); HRMS calc. for C₂₂H₂₄N₂O₁₀ (M⁺) m/z 476.1431, found 476.1425.

- 10 **2-(3-Hydroxypropoxy)-4-nitrobenzyl 4-nitrophenyl carbonate (86).** A solution of ether 85 (207 mg, 0.47 mmol) in THF (20 mL) and 1 M HCl (5 mL) was stirred at 20 °C for 16 h. The solvent was evaporated and the residue partitioned between EtOAc (50 mL) and water (50 mL). The organic fraction was dried, the solvent evaporated, and the residue purified by chromatography, eluting with 50% EtOAc/light petroleum, to give 86 (125 mg, 68%) as a white solid, mp (EtOAc/light petroleum) 116-117 °C; ¹H NMR δ 8.29 (ddd, $J = 9.1, 3.2, 2.1$ Hz, 2 H, H 3, H 5), 7.88 (dd, $J = 8.3, 2.1$ Hz, 1 H, H 5'), 7.80 (d, $J = 2.1$ Hz, 1 H, H 3'), 7.58 (d, $J = 8.3$ Hz, 1 H, H 6'), 7.40 (ddd, $J = 9.1, 3.2, 2.1$ Hz, 2 H, H 2, H 6), 5.41 (s, 2 H, CH₂O), 4.30 (t, $J = 6.0$ Hz, 2 H, CH₂O), 3.90 (dt, $J = 5.4, 4.6$ Hz, 2 H, CH₂O), 2.10-2.15 (m, 2 H, CH₂), 1.65 (br s, 1 H, OH); ¹³C NMR δ 157.0 (C 2'), 155.3 (C 1), 152.3 (OCONH), 149.3 (C 4'), 145.5 (C 4), 129.8 (C 1'), 129.6 (C 6'), 125.4 (C 2, C 6), 121.7 (C 3, C 5), 115.8 (C 5'), 106.4 (C 3'), 66.2 (CH₂O), 65.3 (CH₂O), 59.5 (CH₂O), 31.7 (CH₂); Anal. (C₁₇H₁₆N₂O₉) C, H, N.

- 25 **2-(3-Hydroxypropoxy)-4-nitrobenzyl doxorubicin carbamate (87).** A solution of carbonate 86 (41 mg, 104 μmol) in DMF (2 mL) was added dropwise to a stirred solution of doxorubicin 13 (46 mg, 86 μmol) and Et₃N (15 μL, 104 μmol) in DMF (5 mL) at 20 °C and the solution stirred for 16 h. The solvent was evaporated and the residue purified by chromatography, eluting with a gradient (0-10%) of MeOH/DCM, to give 87 (69 mg, 84%) as a red solid, mp (DCM) 154-160 °C; ¹H NMR [(CD₃)₂SO] δ 14.00 (s, 1 H, 6-OH), 13.24 (s, 1 H, 11-OH), 7.85-7.89 (m, 2 H, H 1, H 3), 7.80 (dd, $J = 8.3, 1.8$ Hz, 1 H, H 5'), 7.71 (d, $J = 1.8$ Hz, 1 H, H 3'), 7.62 (dd, $J = 6.6, 2.8$ Hz, 1 H, H 2), 7.50 (d, $J = 8.3$ Hz, 1 H, H 6'), 7.07 (d, $J = 8.0$ Hz, 1 H, OCONH), 5.42 (s, 1 H, 9-OH), 5.14 (br s, 1 H, H 1'), 5.05 (d, $J =$

- 18.4 Hz, 1 H, CH₂O), 4.99 (d, *J* = 18.4 Hz, 1 H, CH₂O), 4.92 (br s, 1 H, H 7), 4.85 (t, *J* = 6.0 Hz, 1 H, 14-OH), 4.74 (d, *J* = 5.8 Hz, 1 H, 4'-OH), 4.58 (d, *J* = 6.0 Hz, 2 H, H 14), 4.55 (t, *J* = 5.3 Hz, 1 H, H 5'), 4.14-4.20 (m, 2 H, CH₂O), 3.97 (s, 3 H, 4-OCH₃), 3.69-3.76 (m, 1 H, H 3'), 3.54 (dt, *J* = 6.0, 5.7 Hz, 2 H, CH₂O), 3.48 (br s, 1 H, H 4'), 3.30 (br s, 1 H, OH),
- 5 2.98 (d, *J* = 18.2 Hz, 1 H, H 10), 2.90 (d, *J* = 18.2 Hz, 1 H, H 10), 2.22 (br d, *J* = 14.4 Hz, 1 H, H 8), 2.09 (dd, *J* = 14.4, 5.5 Hz, 1 H, H 8), 1.88-1.92 (m, 1 H, H 2'), 1.82-1.87 (m, 2 H, CH₂), 1.50 (dd, *J* = 12.4, 3.7 Hz, 1 H, H 2'), 1.13 (d, *J* = 6.4 Hz, 3 H, H 6'); ¹³C NMR [(CD₃)₂SO] δ 213.7 (C 13), 186.4 (C 5), 186.3 (C 12), 160.7 (C 4), 156.0 (C 2''), 155.9 (C 6), 154.9 (C 11), 154.4 (OCONH), 147.8 (C 4''), 136.1 (C 2), 135.4 (C 12a), 134.5 (C 6a),
- 10 134.0 (C 10a), 133.2 (C 1''), 127.8 (C 6''), 119.9 (C 4a), 119.6 (C 1), 118.9 (C 3), 115.3 (C 5''), 110.6 (C 5a), 110.5 (C 11a), 105.8 (C 3''), 100.2 (C 1'), 74.8 (C 9), 69.8 (C 7), 67.9 (C 4'), 66.6 (C 5'), 65.6 (CH₂O), 63.6 (C 14), 59.8 (CH₂O), 57.0 (CH₂O), 56.5 (4-OCH₃), 47.2 (C 3'), 36.5 (C 8), 32.0 (C 10), 31.7 (CH₂), 29.7 (C 2'), 16.9 (C 6'); MS (FAB⁺) *m/z* 797 (MH⁺, 0.3%); HRMS (FAB⁺) calc. for C₃₈H₄₀N₂O₁₇ (MH⁺) *m/z* 797.2405, found 797.2953;
- 15 Anal. (C₃₈H₄₀N₂O₁₇·½H₂O) C, H, N.

Example 4G. Preparation of 2-(3-hydroxypropoxy)-4-nitrobenzyl bis(3-[(5-methyl-4-acridinyl)carbonyl]amino}propyl)carbamate (91).

4-Nitro-2-[3-(tetrahydro-2H-pyran-2-yloxy)propoxy]benzyl bis(3-[(trifluoroacetyl)amino]propyl)carbamate (88).

- 20 A solution of alcohol 75 (623 mg, 2.0 mmol) and DIEA (0.40 mL, 2.4 mmol) in DCM (8 mL) was added dropwise to a solution of triphosgene (208 mg, 0.70 mmol) in DCM (6 mL) over 30 minutes at 5 °C and stirred for 1 h. The reaction mixture was added dropwise to a suspension of bistrifluoroacetamide
- 25 47 (880 mg, 2.0 mmol) and DIEA (0.76 mL, 4.8 mmol) in DCM (8 mL) and the solution stirred at 20 °C for 16 h. The solvent was evaporated and the residue purified by chromatography, eluting with 50% EtOAc/petroleum ether, to give 88 (804 mg, 61%) as a colorless oil, ¹H NMR [(CD₃)₂SO] δ 7.85 (dd, *J* = 8.0, 2.0 Hz, 1 H, H 5'), 7.80 (br s, 1 H, CONH), 7.76 (d, *J* = 2.0 Hz, 1 H, H 3'), 7.44 (d, *J* = 8.0 Hz, 1 H, H 6'), 6.81 (br s, 1 H, CONH), 5.25 (s, 2 H, CH₂O), 4.58-4.61 (m, 1 H), 4.22-4.26 (m, 2 H), 3.91-3.98 (m, 1 H),
- 30 3.79-3.86 (m, 1 H), 3.56-3.63 (m, 1 H), 3.46-3.53 (m, 1 H), 3.28-3.40 (m, 8 H), 2.04-2.16 (m, 2 H), 1.72-1.87 (m, 6 H), 1.50-1.63 (m, 4 H); ¹³C NMR [(CD₃)₂SO] δ 157.0, 156.9, 148.9, 131.5, 129.4, 115.7, 105.3, 99.2, 65.9, 63.7, 62.6, 62.5, 44.3 (2), 37.4, 36.1, 30.7,

29.4, 28.1, 27.1, 25.3, 19.6, $2 \times \text{CF}_3\text{CO}$ not observed; HRMS (FAB⁺) calc. for $\text{C}_{26}\text{H}_{34}\text{F}_6\text{N}_4\text{O}_9$ (M^+) m/z 660.2230; found 660.2234

4-Nitro-2-[3-(tetrahydro-2H-pyran-3-yloxy)propoxy]benzyl bis(3-[(5-methyl-4-acridinyl)carbonyl]amino}propyl)carbamate (90). A solution of carbamate 88 (165 mg, 0.25 mmol), Cs_2CO_3 (1.0 g, 3.0 mmol) and water (1 mL) in methanol (4 mL) was stirred at 20 °C for 8 h. The pH was adjusted to 10, water (50 mL) added, the solution was extracted with DCM (3×50 mL). The combined organic fraction was dried, and the solvent was evaporated to give crude 4-nitro-2-[3-(tetrahydro-2H-pyran-2-yloxy)propoxy]benzyl bis(3-aminopropyl)carbamate (89). 4-(1H-Imidazol-1-ylcarbonyl)-5-methylacridine (50) [S. A. Gamage, J. A. Spicer, G. J. Atwell, G. J. Finlay, B. C. Baguley, W. A. Denny, *J. Med. Chem.*, 1999, 42, 2383-2393] (144 mg, 0.50 mmol) was added to a solution of carbamate (89) in THF (10 mL) at 5 °C and the reaction mixture was stirred at 20 °C for 8 h. The solvent was evaporated, and the residue was purified by chromatography on alumina-90, eluting with 1%MeOH/55%EtOAc/DCM, to give 90 (183 mg, 88%) as a yellow solid, mp (EtOAc/DCM) 80-81 °C; ¹H NMR δ 11.90 (s, 1 H, NH), 11.83 (s, 1 H, NH), 8.88-8.92 (m, 2 H), 8.62-8.72 (m, 2 H), 8.02-8.05 (m, 2 H), 7.74-7.83 (m, 2 H), 7.50-7.63 (m, 4 H), 7.36-7.45 (m, 2 H), 7.30 (d, $J = 2.0$ Hz, 1 H, H 3''), 7.02 (d, $J = 8.4$ Hz, 1 H, H 6''), 6.90 (dd, $J = 8.4, 2.0$ Hz, 1 H, H 5''), 5.01 (s, 2 H, CH_2O), 4.56 (s, 1 H), 4.00-3.50 (m, 14 H), 2.83 (s, 3 H, CH_3), 2.71 (s, 3 H, CH_3), 2.14-1.50 (m, 12 H); ¹³C NMR δ 166.1, 155.6, 155.5, 147.5 (2), 147.0 (2), 145.2 (2), 137.9 (2), 135.8, 135.4, 135.2 (2), 132.6 (2), 132.3 (2), 131.0 (2), 128.3, 128.0, 126.5, 126.4, 126.2, 126.1 (2), 125.7 (2), 125.3 (2), 114.9, 104.9, 99.1, 65.6, 63.6, 62.5, 61.6, 45.9, 45.1, 37.7, 37.1, 30.7, 29.3, 29.4, 28.6, 25.4, 19.7, 18.9, 18.8, 14.2; Anal. ($\text{C}_{52}\text{H}_{54}\text{N}_6\text{O}_9 \cdot \frac{1}{2}\text{H}_2\text{O}$) C, H, N.

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2-(3-Hydroxypropoxy)-4-nitrobenzyl bis(3-[(5-methyl-4-acridinyl)carbonyl]amino}propyl)carbamate dihydrochloride (91). A solution of ether 90 (51 mg, 56 μmol) and HCl (1 M, 1.5 mL) in MeOH (10 mL) was stirred at 20 °C for 4 hrs. The solvent was evaporated and the residue was recrystallized to give 91 (46 mg, 92%) as a yellow solid, mp (MeOH/EtOAc/light petroleum) 143-145°C; ¹H NMR [$(\text{CD}_3)_2\text{SO}$] δ 11.23 (s, 2 H, $2 \times \text{NH}$), 9.17 (s, 1 H), 9.11 (s, 1 H), 8.70 (br s, 2 H), 8.24 (br s, 2 H), 7.96 (br s, 2 H), 7.66 (br s, 4 H), 7.51 (br s, 2 H), 7.32 (d, $J = 2.0$ Hz, 1 H, H 3''), 7.02 (d, $J = 8.4$

30

Hz, 1 H, H 6"), 6.97 (dd, $J = 8.4, 2.0$ Hz, 1 H, H 5"), 4.80 (s, 2 H, CH₂O), 3.92-3.96 (m, 2 H), 3.50-3.53 (m, 10 H), 2.72 (s, 3 H, CH₃), 2.63 (s, 3 H, CH₃), 2.01-2.04 (m, 4 H, 2 × CH₂), 1.75-1.82 (m, 2 H); HRMS (FAB⁺) calc. for (C₄₇H₄₆N₆O₈) (M⁺) m/z 823.3455, found 823.3467; Anal. (C₄₇H₄₆N₆O₈·2HCl·2½H₂O) C, H, N.

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Example 4H. Preparation of 4-nitro-2-[3-(phosphonooxy)propoxy]benzyl 1-(chloromethyl)-3-[(5,6,7-trimethoxy-1H-indol-2-yl)carbonyl]-2,3-dihydro-1H-benzo[e]indol-5-ylcarbamate (93).

- 2-(3-[[Di(*tert*-butoxy)phosphoryl]oxy]propoxy)-4-nitrobenzyl 1-(chloromethyl)-3-
 10 [(5,6,7-trimethoxy-1H-indol-2-yl)carbonyl]-2,3-dihydro-1H-benzo[e]indol-5-ylcarbamate (92). Tetrazole (40 mg, 567 μmol) was added to a stirred solution of alcohol
 84 (Example 4E) 136 mg, 189 μmol) and di-*tert*-butyl diethylphosphoramidite (68 μL, 227
 mmol) in THF (10 mL) under N₂ and the solution stirred at 20 °C for 4 h. The solution was
 cooled to -40 °C and a dried (Na₂SO₄) solution of MCPBA (70 %, 65 mg, 265 μmol) in
 15 DCM (3 mL) added. The solution was stirred at -40 °C for 10 min and a solution of 10%
 NaHSO₄ (10 mL) added and the mixture stirred for 10 min. The mixture was extracted with
 diethyl ether (80 mL), the organic fraction washed with 10% aq. NaHSO₄ (10 mL), sat. aq.
 KHCO₃ (10 mL), dried, and the solvent evaporated. The residue was purified by
 chromatography, eluting with a gradient (10-50%) EtOAc/light petroleum, to give 92 (160
 20 mg, 93%) as an oil, ¹H NMR δ 9.42 (s, 1 H, indole-NH), 8.89 (s, 1 H, OCONH), 8.15 (d, J
 = 8.5 Hz, 1 H, H 6), 7.83 (dd, $J = 8.2, 2.1$ Hz, 1 H, H 5"), 7.79 (s, 1 H, H 4), 7.75 (d, $J = 8.2$
 Hz, 1 H, H 6"), 7.73 (d, $J = 2.1$ Hz, 1 H, H 3"), 7.60 (d, $J = 8.3$ Hz, 1 H, H 9), 7.53 (ddd, J
 = 8.3, 7.1, 0.8 Hz, 1 H, H 8), 7.39 (ddd, $J = 8.5, 7.1, 0.8$ Hz, 1 H, H 7), 7.01 (d, $J = 2.1$ Hz,
 1 H, H 3'), 6.89 (s, 1 H, H 4'), 5.34 (s, 2 H, CH₂O), 4.81 (dd, $J = 10.7, 1.8$ Hz, 1 H, H 2),
 25 4.64 (dd, $J = 10.7, 8.6$ Hz, 1 H, H 2), 4.35 (dt, $J = 5.6, 5.5$ Hz, 2 H, CH₂O), 4.29 (t, $J = 5.6$
 Hz, 2 H, CH₂O), 4.15-4.20 (m, 1 H, H 1), 4.10 (s, 3 H, OCH₃), 3.98 (dd, $J = 11.2, 2.9$ Hz, 1
 H, CH₂Cl), 3.95 (s, 3 H, OCH₃), 3.92 (s, 3 H, OCH₃), 3.45 (dd, $J = 10.9, 10.8$ Hz, 1 H,
 CH₂Cl), 2.19-2.25 (m, 2 H, CH₂), 1.35 (2s, 18 H, 2 × OC(CH₃)₃); MS (FAB⁺) m/z 913
 (MH⁺, 0.4%), 911 (MH⁺, 0.8); HRMS (FAB⁺) calc. for C₄₄H₅₃³⁵ClN₄O₁₃P (MH⁺) m/z
 30 911.3035, found 911.3003; calc. for C₄₄H₅₃³⁷ClN₄O₁₃P (MH⁺) m/z 913.3006, found
 913.3002.

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4-Nitro-2-[3-(phosphonoxy)propoxy]benzyl 1-(chloromethyl)-3-[(5,6,7-trimethoxy-1*H*-indol-2-yl)carbonyl]-2,3-dihydro-1*H*-benzo[*e*]indol-5-ylcarbamate (93).

- Trifluoroacetic acid (130 μ L, 1.64 μ mol) was added to a stirred solution of ester 92 (150 mg, 165 μ mol) in DCM (5 mL) and the solution stirred at 20 °C for 1 h. The solvent was
- 5 evaporated, and the residue azeotroped with benzene (3 \times 1 mL) to give 93 (88 mg, 66%) as a gum, ¹H NMR δ 11.47 (s, 1 H, indole-NH), 9.94 (s, 1 H, OCONH), 8.57 (br s, 3 H, H 4, 2 \times OH), 8.11 (d, J = 8.5 Hz, 1 H, H 6), 7.98 (d, J = 8.3 Hz, 1 H, H 9), 7.91 (d, J = 8.3 Hz, 1 H, H 5"), 7.81 (d, J = 1.8 Hz, 1 H, H 3"), 7.69 (d, J = 8.3 Hz, 1 H, H 6"), 7.58 (ddd, J = 8.3, 7.2, 0.7 Hz, 1 H, H 8), 7.47 (ddd, J = 8.5, 7.2, 0.7 Hz, 1 H, H 7), 7.10 (d, J = 2.2 Hz,
- 10 1 H, H 3'), 6.97 (s, 1 H, H 4'), 5.29 (s, 2 H, CH₂O), 4.80 (dd, J = 10.8, 9.4 Hz, 1 H, H 2), 4.53 (dd, J = 10.8, 1.7 Hz, 1 H, H 2), 4.31–4.37 (m, 1 H, H 1), 4.27 (t, J = 6.1 Hz, 2 H, CH₂O), 4.07 (dd, J = 11.2 Hz, 1 H, CH₂Cl), 4.03 (dt, J = 7.1, 6.2 Hz, 2 H, CH₂O), 3.91–3.95 (m, 4 H, OCH₃, CH₂Cl), 3.82 (s, 3 H, OCH₃), 3.80 (s, 3 H, OCH₃), 2.02–2.10 (m, 2 H, CH₂); ¹³C NMR δ 160.2 (CO), 156.2 (C 2"), 154.4 (OCONH), 149.1 (C 5'), 148.1 (C 4"), 141.4 (C
- 15 3a), 139.9 (C 6'), 139.0 (C 7'), 134.4 (C 5), 132.8 (C 1"), 130.8 (C 9a), 129.4 (C 2'), 128.5 (C 6"), 127.1 (C 8), 125.4 (C 5a, C 7a'), 124.3 (C 7), 123.8 (C 9), 123.2 (C 6), 123.1 (C 3a'), 122.0 (C 9b), 115.4 (C 5"), 113.0 (C 4), 106.2 (C 3'), 106.0 (C 3"), 98.0 (C 4'), 65.1 (CH₂O), 61.3 (CH₂O), 61.0 (OCH₃), 60.9 (OCH₃), 60.7 (CH₂O), 55.9 (OCH₃), 54.9 (C 2), 47.5 (CH₂Cl), 41.2 (C 1), 29.6 (CH₂); MS (FAB⁺) m/z 801 (MH⁺, 0.5%), 799 (MH⁺, 0.8);
- 20 HRMS (FAB⁺) calc. for C₃₆H₃₇³⁵ClN₄O₁₃P (MH⁺) m/z 799.1783, found 799.1757; calc. for C₃₆H₃₇³⁷ClN₄O₁₃P (MH⁺) m/z 801.1754, found 801.1730.

Example 4I. Preparation of 2-(2,3-dihydroxypropoxy)-4-nitrobenzyl 1-(chloromethyl)-

- 25 **3-[(5,6,7-trimethoxy-1*H*-indol-2-yl)carbonyl]-2,3-dihydro-1*H*-benzo[*e*]indol-5-ylcarbamate (99).**

- Methyl 4-nitro-2-(2-oxiranylmethoxy)benzoate (94).** A mixture of methyl 4-nitrosalicylate (66) (0.99 g, 5.02 mmol) and K₂CO₃ (1.04 g, 7.53 mmol) in DMF (25 mL) was stirred at 20 °C for 20 min. Epichlorohydrin (0.59 mL, 7.53 mmol) was added and the
- 30 mixture stirred at 100 °C for 2 h. The mixture was poured into water, extracted with EtOAc (3 \times 100 mL), the combined organic extracts washed with water (2 \times 50 mL), brine (50 mL), dried, and the solvent evaporated. The residue was purified by chromatography, eluting with a gradient (20–50%) EtOAc/light petroleum, to give (i) starting material (0.18

g, 18%) and (ii) **94** (0.75 g, 59 %) as a colourless solid, mp (EtOAc/light petroleum) 62-63 °C; ¹H NMR δ 7.91 (dd, *J* = 7.7, 1.0 Hz, 1 H, H 5), 7.84-7.86 (m, 2 H, H 3, H 6), 4.49 (dd, *J* = 11.2, 2.4 Hz, 1 H, H 3'), 4.14 (dd, *J* = 11.2, 5.2 Hz, 1 H, H 3'), 3.94 (s, 3 H, OCH₃), 3.40-3.44 (m, 1 H, H 2'), 2.91-2.97 (m, 2 H, H 1'); ¹³C NMR δ 165.0 (CO₂), 158.1 (C 2), 150.6 (C 4), 132.3 (C 6), 126.1 (C 1), 115.6 (C 5), 108.4 (C 3), 69.6 (OCH₃), 52.6 (CH₂O), 49.7 (CH₂O), 44.3 (C 2'); MS (CI, NH₃) *m/z* 295 (M+CH₃CN⁺, 70%), 259 (MH⁺, 100%); Anal. (C₁₁H₁₁NO₆) C, H, N.

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Methyl 2-(2,3-dihydroxypropoxy)-4-nitrobenzoate (95). Perchloric acid (1 mL) and water (3 mL) was added to a stirred solution of **94** (205 mg, 0.81 mmol) in THF (20 mL) and the solution stirred at 20 °C for 16 h. The solvent was evaporated and the residue partitioned between EtOAc (50 mL) and water (50 mL). The organic fraction was washed with water (50 mL), brine (25 mL), dried, and the solvent evaporated. The residue was purified by chromatography, eluting with 70% EtOAc/light petroleum, to give **95** (172 mg, 78%) as an oil which solidified on standing, mp 60-65 °C; ¹H NMR δ 8.02 (d, *J* = 8.5 Hz, 1 H, H 6), 7.87 (dd, *J* = 8.5, 2.0 Hz, 1 H, H 5), 7.84 (d, *J* = 2.0 Hz, 1 H, H 3), 4.38 (dd, *J* = 9.3, 5.4 Hz, 1 H, H 3'), 4.23 (dd, *J* = 9.3, 5.4 Hz, 1 H, H 3'), 4.10-4.14 (m, 1 H, H 2'), 3.95 (s, 3 H, OCH₃), 3.88 (br d, *J* = 4.1 Hz, 2 H, H 1'), 3.05 (br s, 1 H, OH), 1.95 (br s, 1 H, OH); ¹³C NMR δ 164.8 (CO₂), 159.1 (C 2), 151.0 (C 4), 132.8 (C 6), 124.9 (C 1), 115.6 (C 5), 108.8 (C 3), 73.0 (CH₂O), 69.2 (C 2'), 63.2 (CH₂O), 52.8 (OCH₃); MS (CI, NH₃) *m/z* 272 (MH⁺, 1%), 240 (50%), 165 (100); HRMS (CI, NH₃) calc. for C₁₁H₁₄NO₇ (MH⁺) *m/z* 272.0770, found 272.0766. Anal. (C₁₁H₁₃NO₇) C, H, N.

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Methyl 2-[(2,2-dimethyl-1,3-dioxolan-4-yl)methoxy]-4-nitrobenzoate (96). 2,2-Dimethoxypropane (0.91 mL, 7.37 mmol) was added dropwise to a stirred solution of diol **95** (400 mg, 1.47 mmol) and PPTS (37 mg, 0.15 mmol) in DMF (20 mL) under N₂ and stirred at 20 °C for 24 h. The solvent was evaporated and the residue partitioned between EtOAc (100 mL) and water (100 mL). The organic fraction was washed with water (50 mL), brine (25 mL), dried, and the solvent evaporated. The residue was purified by chromatography, eluting with 30% EtOAc/light petroleum, to give **96** (458 mg, 100%) as a yellow oil, ¹H NMR δ 7.90 (d, *J* = 8.2 Hz, 1 H, H 6), 7.84-7.88 (m, 2 H, H 3, H 5), 4.49-4.54 (m, 1 H, H 4'), 4.25 (dd, *J* = 9.6, 4.6 Hz, 1 H, H 5'), 4.19 (dd, *J* = 8.5, 6.4 Hz, 1 H, H 2'), 4.15 (dd, *J* = 9.6, 4.6 Hz, 1 H, H 5'), 4.03 (dd, *J* = 8.5, 5.8 Hz, 1 H, H 2'), 3.94 (s, 3 H, OCH₃), 1.46 (s, 3 H, CH₃), 1.41 (s, 3 H, CH₃); ¹³C NMR δ 165.1 (CO₂), 158.2 (C 2), 150.6

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- 76 -

(C 4), 132.2 (C 6), 126.4 (C 1), 115.5 (C 5), 109.9 (C 2"), 108.4 (C 3), 73.6 (C 4"), 69.8 (CH₂O), 66.5 (CH₂O), 52.6 (OCH₃), 26.6 (CH₃), 25.3 (CH₃); MS (CI, NH₃) *m/z* 312 (MH⁺, 15%), 296 (95), 101 (95), 71 (100); HRMS (CI, NH₃) calc. for C₁₄H₁₈NO₇ (MH⁺) *m/z* 312.1083, found 312.1092.

5

{2-[(2,2-Dimethyl-1,3-dioxolan-4-yl)methoxy]-4-nitrophenyl}methanol (97). DIBALH (1 M in DCM, 5.1 mL, 5.1 mmol) was added to a stirred solution of ester 96 (457 mg, 1.47 mmol) in THF (50 mL) at 5 °C and the solution stirred at 5 °C for 1 h. The solution was poured into a solution of potassium sodium tartrate (1 M, 100 mL) and the mixture stirred vigorously for 20 min. The mixture was extracted with EtOAc (3 × 50 mL), the combined organic fraction washed with water (50 mL), brine (50 mL), dried, and the solvent evaporated. The residue was purified by chromatography, eluting with 20% EtOAc/light petroleum, to give 97 (385 mg, 92%) as a white solid, mp (EtOAc/light petroleum) 90-92 °C; ¹H NMR δ 7.87 (dd, *J* = 8.2, 2.1 Hz, 1 H, H 5), 7.72 (d, *J* = 2.1 Hz, 1 H, H 3), 7.50 (d, *J* = 8.2 Hz, 1 H, H 6), 4.82 (d, *J* = 14.1 Hz, 1 H, CH₂O), 4.70 (d, *J* = 14.1 Hz, 1 H, CH₂O), 4.51-4.57 (m, 1 H, H 4"), 4.23 (dd, *J* = 9.8, 4.0 Hz, 1 H, H 5"), 4.19 (dd, *J* = 9.8, 5.4 Hz, 1 H, H 2"), 4.11 (dd, *J* = 9.8, 5.4 Hz, 1 H, H 5"), 3.95 (dd, *J* = 8.7, 5.4 Hz, 1 H, H 2'), 3.25 (br s, 1 H, OH), 1.48 (s, 3 H, CH₃), 1.41 (s, 3 H, CH₃); ¹³C NMR δ 156.5 (C 2), 148.2 (C 4), 137.2 (C 1), 128.6 (C 6), 116.6 (C 5), 110.0 (C 2"), 106.4 (C 3), 73.7 (CH₂O), 69.7 (CH₂O), 66.0 (CH₂O), 61.0 (CH₂O), 26.6 (CH₃), 25.0 (CH₃); MS *m/z* 283 (M⁺, 3%), 268 (20), 225 (30), 101 (100); HRMS calc for C₁₃H₁₇NO₆ (M⁺) *m/z* 283.1056, found 283.1055; Anal. (C₁₃H₁₇NO₆) C, H, N.

2-[(2,2-Dimethyl-1,3-dioxolan-4-yl)methoxy]-4-nitrobenzyl 1-(chloromethyl)-3-[(5,6,7-trimethoxy-1*H*-indol-2-yl)carbonyl]-2,3-dihydro-1*H*-benzo[*e*]indol-5-ylcarbamate (98). A solution of triphosgene (22 mg, 75 μmol) in DCM (3 mL) was added dropwise to a stirred solution of amin 1 [G. J. Atwell, W. R. Wilson, W. A. Denny, *Bioorg. Med. Chem. Lett.*, 1997, 7, 1483] (100 mg, 215 μmol) and Et₃N (60 μL, 429 μmol) in DCM (10 mL) and stirred at °C for 2 h. A solution of alcohol 97 (73 mg, 256 μmol) in DCM (3 mL) was added, followed by nBu₂Sn(OAc)₂ (2 drops) and the solution stirred at 20 °C for 24 h. The solvent was evaporated and the residue purified by chromatography, eluting with 20%EtOAc/DCM, to give 98 (160 mg, 96%) as a gum; ¹H NMR δ 9.44 (s, 1 H, indole-NH), 8.94 (s, 1 H, OCONH), 7.92 (d, *J* = 8.5 Hz, 1 H, H 6), 7.87 (dd, *J* = 8.2, 2.1 Hz, 1 H,

H 5"), 7.81 (d, $J = 8.2$ Hz, 1 H, H 9), 7.73 (d, $J = 2.1$ Hz, 1 H, H 3"), 7.58 (ddd, $J = 8.2, 7.3, 0.7$ Hz, 1 H, H 8), 7.45-7.51 (m, 2 H, H 7, H 6"), 7.13 (br s, 1 H, H 4), 7.02 (d, $J = 2.2$ Hz, 1 H, H 3'), 6.89 (s, 1 H, H 4'), 5.38 (s, 2 H, CH₂O), 4.83 (dd, $J = 10.8, 1.7$ Hz, 1 H, H 2), 4.69 (dd, $J = 10.8, 8.7$ Hz, 1 H, H 2), 4.50-4.56 (m, 1 H, H 4'''), 4.23 (dd, $J = 9.8, 4.0$ Hz, 1 H, H 5'''), 4.15-4.20 (m, 2 H, H 1, H 2''), 4.09-4.14 (m, 4 H, OCH₃, H 5'''), 3.95-4.00 (m, 5 H, OCH₃, CH₂Cl, H 2''), 3.92 (s, 3 H, OCH₃), 3.50 (dd, $J = 10.9, 10.8$ Hz, 1 H, CH₂Cl), 1.45 (s, 3 H, CH₃), 1.39 (s, 3 H, CH₃); MS (FAB⁺) m/z 777 (MH⁺, 10%), 775 (MH⁺, 35); HRMS (FAB⁺) calc. for C₃₉H₄₀³⁵CIN₄O₁₁ (MH⁺) m/z 775.2381, found 777.2379; calc. for C₃₉H₄₀³⁷CIN₄O₁₁ (MH⁺) m/z 777.2535, found 777.2354.

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2-(2,3-Dihydroxypropoxy)-4-nitrobenzyl 1-(chloromethyl)-3-[(5,6,7-trimethoxy-1H-indol-2-yl)carbonyl]-2,3-dihydro-1H-benzo[e]indol-5-ylcarbamate (99). 1 M HCl (1 mL) was added to a stirred suspension of 98 (160 mg, 206 μ mol) in THF (20 mL) and the mixture stirred at 20 °C for 16 h. The mixture was evaporated and the residue partitioned

15 between DCM (50 mL) and water (50 mL). The organic fraction was washed with water (30 mL), brine (30 mL), dried, and the solvent evaporated. The residue was purified by chromatography, eluting with 50%EtOAc/DCM, to give 99 (87 mg, 56%) as a white solid, mp (MeOH/iPr₂O) 147-149 °C; ¹H NMR [(CD₃)₂SO] δ 11.46 (s, 1 H, indole-NH), 9.90 (s, 1 H, OCONH), 8.56 (s, 1 H, H 4), 8.12 (d, $J = 8.5$ Hz, 1 H, H 6), 7.98 (d, $J = 8.3$ Hz, 1 H, H 9), 7.92 (dd, $J = 8.3, 1.9$ Hz, 1 H, H 5"), 7.83 (d, $J = 1.9$ Hz, 1 H, H 3"), 7.69 (d, $J = 8.3$ Hz, 1 H, H 6"), 7.59 (dd, $J = 8.2, 7.6$ Hz, 1 H, H 8), 7.49 (dd, $J = 8.5, 7.6$ Hz, 1 H, H 7), 7.09 (d, $J = 2.1$ Hz, 1 H, H 3'), 6.98 (s, 1 H, H 4'), 5.33 (s, 2 H, CH₂O), 5.07 (d, $J = 5.2$ Hz, 1 H, OH), 4.81 (dd, $J = 11.0, 9.7$ Hz, 1 H, H 2), 4.73 (t, $J = 5.7$ Hz, 1 H, H 3'''), 4.53 (dd, $J = 11.0, 3.5$ Hz, 1 H, H 2), 4.32-4.37 (m, 1 H, H 1), 4.24 (dd, $J = 10.0, 3.9$ Hz, 1 H, CH₂Cl), 25 4.09-4.13 (m, 1 H, H 2'''), 4.02-4.06 (m, 1 H, H 3'), 3.93-3.96 (m, 4 H, OCH₃, CH₂Cl), 3.84-3.89 (m, 1 H, OH), 3.83 (s, 3 H, OCH₃), 3.81 (s, 3 H, OCH₃), 3.51 (t, $J = 5.7$ Hz, 2 H, H 1''); ¹³C NMR [(CD₃)₂SO] δ 160.2 (CO), 156.2 (C 2"), 154.3 (OCONH), 149.2 (C 5'), 148.0 (C 4"), 141.5 (C 3a), 139.9 (C 6'), 139.0 (C 7'), 134.3 (C 5), 133.0 (C 1"), 130.7 (C 9a), 129.4 (C 2'), 128.0 (C 6"), 127.1 (C 8), 125.4 (C 5a, C 7a), 124.3 (C 7), 123.9 (C 9), 30 123.3 (C 6), 123.1 (C 3a'), 122.0 (C 9b), 115.4 (C 5"), 113.0 (C 4), 106.3 (C 3'), 106.2 (C 3"), 98.0 (C 4'), 70.7 (CH₂O), 69.7 (CHOH), 62.4 (CH₂O), 61.0 (OCH₃), 60.9 (OCH₃), 60.7 (CH₂O), 55.9 (OCH₃), 54.9 (C 2), 47.5 (CH₂Cl), 41.4 (C 1); MS (FAB⁺) m/z 737 (MH⁺, 3%), 735 (MH⁺, 8); HRMS (FAB⁺) calc. for C₃₆H₃₆³⁵CIN₄O₁₁ (MH⁺) m/z 735.2069, found

735.2050; calc. for $C_{36}H_{36}^{37}ClN_4O_{11}$ (MH^+) m/z 737.2040, found 737.2000; Anal. ($C_{36}H_{35}ClN_4O_{11} \cdot CH_3OH$) C, H, N.

Example 4J. Preparation of 2-[3-(dimethylamino)propoxy]-4-nitrobenzyl 1-

5 **(chloromethyl)-3-[(5,6,7-trimethoxy-1H-indol-2-yl)carbonyl]-2,3-dihydro-1H-benzo[e]indol-5-ylcarbamate (102).**

Methyl 2-[3-(dimethylamino)propoxy]-4-nitrobenzoate (100). A mixture of methyl 2-hydroxy-4-nitrobenzoate (66) (1.03 g, 5.22 mmol) and K_2CO_3 (2.17 g, 15.67 mmol) in DMF (30 mL) was stirred at 20 °C for 30 min. A solution of *N*-(3-chloropropyl)-*N,N*-dimethylamine (1.24 g, 7.83 mmol) in DMF (10 mL) was added and the mixture stirred at 100 °C for 3 h. The mixture was poured into water (300 mL), extracted with EtOAc (3 × 100 mL) and the combined organic extract washed with water (2 × 100 mL), brine (50 mL), dried and the solvent evaporated. The residue was purified by chromatography, eluting with a gradient (20-50%) of EtOAc/light petroleum, to give **100** (1.05 g, 71%) as a pale yellow 15 oil which was stored as the HCl salt, mp (EtOAc) 175-177 °C; 1H NMR [$(CD_3)_2SO$] δ 10.90 (br s, 1 H, NHCl), 7.87-7.93 (m, 3 H, H 3, H 5, H 6), 4.32 (t, J = 6.0 Hz, 2 H, CH_2O), 3.89 (s, 3 H, OCH_3), 3.18-3.23 (m, 2 H, CH_2N), 2.77 (d, J = 4.8 Hz, 6 H, $N(CH_3)_2$), 2.18-2.24 (m, 2 H, CH_2); ^{13}C NMR [$(CD_3)_2SO$] δ 165.0 (CO_2), 157.3 (C 2), 150.2 (C 4), 131.6 (C 6), 126.0 (C 1), 115.3 (C 5), 108.4 (C 3), 66.5 (CH_2O), 53.7 (CH_2N), 52.6 (OCH_3), 42.0 20 ($N(CH_3)_2$), 23.3 (CH_2); Anal. ($C_{13}H_{19}ClN_2O_5$) C, H, N, Cl.

{2-[3-(Dimethylamino)propoxy]-4-nitrophenyl}methanol (101). DIBALH (1 M, in DCM, 13.0 mL, 13.0 mmol) was added to a stirred solution of ester **100** (1.05 g, 3.72 mmol) in THF (50 mL) at 5 °C and the solution stirred at 5 °C for 1 h. The solution was 25 poured into a solution of potassium sodium tartrate (1 M, 100 mL) and the mixture stirred vigorously for 20 min. The mixture was extracted with EtOAc (3 × 100 mL), the combined organic fraction washed with water (100 mL), brine (50 mL), dried, and the solvent evaporated. The residue was purified by chromatography on alumina, eluting with a gradient (0-10%) of MeOH/EtOAc, to give **101** (0.81 g, 86%) as a pale yellow solid, mp 30 (EtOAc) 104-105 °C; 1H NMR δ 7.87 (dd, J = 8.3, 2.1 Hz, 1 H, H 5), 7.69 (d, J = 2.1 Hz, 1 H, H 3), 7.64 (d, J = 8.3 Hz, 1 H, H 6), 5.43 (br s, 1 H, OH), 4.58 (s, 2 H, CH_2O), 4.14 (t, J = 6.5 Hz, 2 H, CH_2O), 2.36 (t, J = 7.0 Hz, 2 H, CH_2N), 2.15 (s, 6 H, $N(CH_3)_2$), 1.85-1.91 (m, 2 H, CH_2); ^{13}C NMR δ 155.2 (C 2), 147.0 (C 4), 138.9 (C 1), 126.7 (C 6), 115.3 (C 5),

105.2 (C 3), 66.5 (CH₂O), 57.6 (CH₂O), 55.5 (NCH₂), 45.1 (N(CH₃)₂), 26.5 (CH₂); Anal. (C₁₂H₁₈N₂O₄) C, H, N.

- 2-[3-(Dimethylamino)propoxy]-4-nitrobenzyl 1-(chloromethyl)-3-[(5,6,7-trimethoxy-
5 1*H*-indol-2-yl)carbonyl]-2,3-dihydro-1*H*-benzo[*e*]indol-5-ylcarbamate (102). A
solution of triphosgene (17 mg, 55 μmol) in DCM (2 mL) was added dropwise to a stirred
solution of amine 1 [G. J. Atwell, W. R. Wilson, W. A. Denny, *Bioorg. Med. Chem. Lett.*,
1997, 7, 1483] (65 mg, 140 μmol) and Et₃N (44 μL, 313 μmol) in DCM (10 mL) and
stirred at 20 °C for 2 h. A solution of alcohol 101 (44 mg, 172 μmol) in DCM (2 mL) was
10 added, followed by nBu₂Sn(OAc)₂ (2 drops) and the solution stirred at 20 °C for 24 h. The
solvent was evaporated and the residue purified by chromatography, eluting with a gradient
(0-20%) MeOH/EtOAc, to give 102 (57 mg, 55%) as a yellow solid which was converted
to the hydrochloride salt, mp (MeOH) 176-180 °C; ¹H NMR [(CD₃)₂SO] δ 11.43 (s, 1 H,
indole-NH), 10.47 (br s, 1 H, NH⁺Cl⁻), 9.92 (s, 1 H, OCONH), 8.58 (s, 1 H, H 4), 8.10 (d, *J*
15 = 8.5 Hz, 1 H, H 6), 7.97 (d, *J* = 8.3 Hz, 1 H, H 9), 7.93 (dd, *J* = 8.4, 2.0 Hz, 1 H, H 5"),
7.82 (d, *J* = 2.0 Hz, 1 H, H 3"), 7.71 (br d, *J* = 8.4 Hz, 1 H, H 6"), 7.56-7.61 (m, 1 H, H 8),
7.46-7.51 (m, 1 H, H 7), 7.10 (d, *J* = 2.1 Hz, 1 H, H 3'), 6.97 (s, 1 H, H 4'), 5.34 (s, 2 H,
CH₂O), 4.81 (dd, *J* = 10.8, 9.5 Hz, 1 H, H 2), 4.53 (dd, *J* = 10.8, 1.7 Hz, 1 H, H 2), 4.33-
4.38 (m, 1 H, H 1), 4.30 (t, *J* = 5.9 Hz, 2 H, CH₂O), 4.07 (dd, *J* = 11.1, 2.9 Hz, 1 H, CH₂Cl),
20 3.94-3.97 (m, 4 H, CH₂Cl, OCH₃), 3.83 (s, 3 H, OCH₃), 3.81 (s, 3 H, OCH₃), 3.23-3.27 (t, *J*
= 7.5 Hz, 2 H, CH₂N), 2.75 (s, 6 H, N(CH₃)₂), 2.17-2.23 (m, 2 H, CH₂); ¹³C NMR
[(CD₃)₂SO] δ 160.2 (CO), 155.8 (C 2"), 154.3 (OCONH), 149.2 (C 5'), 148.0 (C 4"), 141.5
(C 3a), 139.9 (C 6'), 138.9 (C 7'), 134.3 (C 5), 132.9 (C 1"), 130.7 (C 2'), 129.5 (C 9a),
128.5 (C 6"), 127.2 (C 8), 125.4 (C 7a'), 124.4 (C 7), 123.7 (C 9), 123.3 (C 6), 123.1 (C
25 3a'), 122.1 (C 9b), 121.2 (C 5a), 115.7 (C 5"), 113.0 (C 4), 106.3 (C 3', C 3"), 98.0 (C 4'),
66.8 (CH₂O), 61.0 (OCH₃), 60.9 (OCH₃), 60.7 (CH₂O), 55.9 (OCH₃), 54.9 (C 2), 53.7
(CH₂N), 47.5 (CH₂Cl), 42.0 (N(CH₃)₂), 41.1 (C 1), 23.6 (CH₂); Anal. (C₃₈H₄₁ClN₅O₉·2HCl):
C, H, N.

- 30 **Example 4K. Preparation of 2-[3-(4-morpholinyl)propoxy]-4-nitrobenzyl 1-
(chloromethyl)-3-[(5,6,7-trimethoxy-1*H*-indol-2-yl)carbonyl]-2,3-dihydro-1*H*-
benzo[*e*]indol-5-ylcarbamate (105).**
Methyl 2-[3-(4-morpholinyl)propoxy]-4-nitrobenzoate (103). A mixture of methyl 2-

hydroxy-4-nitrobenzoate (1.0 g, 5.12 mmol) and K_2CO_3 (1.06 g, 7.68 mmol) in DMF (20 mL) was stirred at 20 °C for 30 min. A solution of 4-(3-chloropropyl)morpholine (0.98 g, 7.68 mmol) in DMF (5 mL) was added and the mixture stirred at 100 °C for 6 h. The mixture was cooled to 20 °C and poured into water (300 mL) and extracted with EtOAc (3 × 100 mL). The combined organic fraction was washed with water (2 × 50 mL), brine (50 mL), dried, and the solvent evaporated. The residue was purified by chromatography, eluting with EtOAc, to give **103** (1.33 g, 80%) as an oil, 1H NMR δ 7.88 (d, J = 9.1 Hz, 1 H, H 6), 7.81 (m, 2 H, H 3, H 5), 4.21 (t, J = 6.3 Hz, 2 H, CH_2O), 3.93 (s, 3 H, OCH_3), 3.69-3.74 (m, 4 H, 2 × CH_2O), 2.57 (t, J = 7.0 Hz, 2 H, CH_2N), 2.47-2.51 (m, 4 H, 2 × CH_2N), 2.02-2.07 (m, 2 H, CH_2); ^{13}C NMR δ 165.3 (CO_2), 158.6 (C 2), 150.6 (C 4), 132.0 (C 6), 126.2 (C 1), 114.8 (C 5), 107.8 (C 3), 67.6 (CH_2O), 66.9 (2 × CH_2O), 55.0 (CH_2N), 53.7 (2 × CH_2N), 52.5 (OCH_3), 25.9 (CH_2). Compound **103** was conveniently stored as the hydrochloride salt, mp (EtOAc) 160-163 °C; Anal. ($C_{15}H_{20}N_2O_6 \cdot HCl$) C, H, Cl, N, calc. 7.8, found 9.1%.

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[2-[3-(4-Morpholinyl)propoxy]-4-nitrophenyl]methanol (104). A solution of ester **103** (1.33 g, 4.10 mmol) in THF (100 mL) was added dropwise to a stirred solution of DIBALH (1 M in DCM, 13.5 mL, 13.5 mmol) at 5 °C and the solution stirred at 5 °C for 1 h. The solution was carefully poured into 1 M HCl (50 mL) and stirred for 10 min. The solution was concentrated under reduced pressure, neutralised and extracted with EtOAc (3 × 100 mL). The combined organic fraction was dried and the solvent evaporated. The residue was purified by chromatography on alumina, eluting with a gradient (0-10%) MeOH/EtOAc, to give **104** (1.07 g, 88%) as a tan solid, mp (EtOAc) 105-106 °C; 1H NMR δ 7.83 (dd, J = 8.2, 2.1 Hz, 1 H, H 5), 7.70 (d, J = 2.1 Hz, 1 H, H 3), 7.46 (d, J = 8.2 Hz, 1 H, H 6), 4.71 (s, 2 H, CH_2O), 4.18 (t, J = 6.0 Hz, 2 H, CH_2O), 3.74-3.77 (m, 4 H, 2 × CH_2O), 2.56 (dd, J = 6.7, 6.5 Hz, 2 H, CH_2N), 2.45-2.49 (m, 4 H, 2 × CH_2N), 2.01-2.06 (m, 2 H, CH_2); ^{13}C NMR δ 156.7 (C 2), 148.2 (C 4), 137.2 (C 1), 128.2 (C 6), 116.1 (C 5), 106.2 (C 3), 67.7 (CH_2O), 66.5 (2 × CH_2O), 60.7 (CH_2O), 56.3 (CH_2N), 53.9 (2 × CH_2N), 25.5 (CH_2); Anal. ($C_{14}H_{20}N_2O_5$) C, H, N.

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2-[3-(4-Morpholinyl)propoxy]-4-nitrobenzyl 1-(chloromethyl)-3-[(5,6,7-trimethoxy-1H-indol-2-yl)carbonyl]-2,3-dihydro-1H-benzo[e]indol-5-ylcarbamate (105). A solution of triphosgene (15.5 mg, 52 μ mol) in DCM (2 mL) was added dropwise to a stirred

solution of amine 1 [G. J. Atwell, W. R. Wilson, W. A. Denny, *Bioorg. Med. Chem. Lett.*, 1997, 7, 1483] (52 mg, 133 μ mol) and Et₃N (42 μ L, 299 μ mol) in DCM (10 mL) and stirred at °C for 2 h. A solution of alcohol 104 (49 mg, 164 μ mol) in DCM (2 mL) was added, followed by nBu₂Sn(OAc)₂ (2 drops) and the solution stirred at 20 °C for 24 h. The solvent was evaporated and the residue purified by chromatography, eluting with a gradient (0-10%) MeOH/EtOAc, to give 105 (96 mg, 92%) as a tan powder mp (EtOAc) 102-107 °C; ¹H NMR δ 9.48 (s, 1 H, indole-NH), 8.93 (s, 1 H, OCONH), 7.91 (d, *J* = 8.5 Hz, 1 H, H 6), 7.78-7.84 (m, 2 H, H 9, H 5"), 7.73 (d, *J* = 1.8 Hz, 1 H, H 3"), 7.57 (ddd, *J* = 8.2, 7.0, 1.0 Hz, 1 H, H 8), 7.50-7.53 (m, 1 H, H 6"), 7.45 (ddd, *J* = 8.5, 7.0, 1.0 Hz, 1 H, H 7), 7.28 (br s, 1 H, H 4), 7.00 (d, *J* = 2.2 Hz, 1 H, H 3'), 6.88 (s, 1 H, H 4'), 5.36 (s, 2 H, CH₂O), 4.81 (dd, *J* = 10.8, 1.8 Hz, 1 H, H 2), 4.68 (dd, *J* = 10.8, 8.7 Hz, 1 H, H 2), 4.16-4.22 (m, 3 H, CH₂O, CH₂Cl), 4.08 (s, 3 H, OCH₃), 3.93-3.99 (m, 4 H, H 1, OCH₃), 3.92 (s, 3 H, OCH₃), 3.69 (t, *J* = 4.6 Hz, 4 H, 2 \times CH₂O), 3.49 (t, *J* = 10.9 Hz, 1 H, CH₂Cl), 2.55 (t, *J* = 7.1 Hz, 2 H, CH₂N), 2.43-2.49 (m, 4 H, 2 \times CH₂N), 2.00-2.08 (m, 2 H, CH₂); ¹³C NMR δ 160.4 (CO), 156.7 (C 2"), 154.0 (OCONH), 150.2 (C 5'), 148.6 (C 4"), 141.7 (C 3a), 140.6 (C 6'), 138.9 (C 7'), 133.9 (C 5), 132.2 (C 1"), 129.8 (C 2'), 129.6 (C 9a), 128.8 (C 6"), 127.5 (C 8), 125.7 (C 7a'), 125.0 (C 7), 123.6 (C 3a'), 123.2 (C 9), 122.4 (C 6, C 9b), 121.6 (C 5a), 115.7 (C 5"), 112.2 (C 4), 106.5 (C 3'), 106.1 (C 3"), 97.7 (C 4'), 67.1 (CH₂O), 66.8 (2 \times CH₂O), 61.9 (CH₂O), 61.5 (OCH₃), 61.1 (OCH₃), 56.3 (OCH₃), 55.2 (C 2), 54.9 (CH₂N), 53.6 (2 \times CH₂N), 45.8 (CH₂Cl), 43.4 (C 1), 26.1 (CH₂); MS (FAB⁺) *m/z* 788 (MH⁺, 6%), 790 (MH⁺, 3); HRMS (FAB⁺) calc. for C₄₀H₄₃³³CIN₅O₁₀ (MH⁺) *m/z* 788.2699, found 788.2721; calc. for C₄₀H₄₃³⁷CIN₅O₁₀ (MH⁺) *m/z* 790.2699, found 790.2728; Anal. (C₄₀H₄₂CIN₅O₁₀·½H₂O) C, H, N.

Example 4L. Preparation of 2-[3-(4-morpholinyl)propoxy]-4-nitrobenzyl bis(3-[(5-methyl-4-acridinyl)carbonyl]amino)propyl)carbamate (107). DIEA (0.3 mL, 3 mmol) was added to a suspension of carbamate dihydrochloride 91 (44 mg, 0.5 mmol) in DCM (10 mL) at 5 °C and the mixture stirred for 10 min. Methanesulfonyl chloride (0.1 mL), was added and the mixture stirred for 30 min. MeOH (2 mL) was added, the mixture stirred for 10 min, and the solvent evaporated. The residue was purified by chromatography on alumina-90, eluting with 1%MeOH/40%EtOAc/DCM to give the crude mesylate 106 (42 mg, 95%) as a yellow solid, ¹H NMR δ 11.94 (s, 1 H, NH), 11.85 (s, 1 H, NH), 8.94 (m, 2 H), 8.77 (m, 2 H), 8.07 (m, 2 H), 7.81 (m, 2 H), 7.62 (m, 4 H), 7.43 (m, 2 H), 7.30 (d, *J* =